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# Development of a first trimester prediction model for preeclampsia and prospective validation in a multicentre setting

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# DEVELOPMENT OF A FIRST TRIMESTER PREDICTION MODEL FOR PREECLAMPSIA AND PROSPECTIVE VALIDATION IN A MULTICENTRE SETTING

Submitted by

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Thesis submitted for the degree of Doctor of Medicine (Research)

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**Supervisors of research**

Dr Liona C. Poon

Professor Kypros H. Nicolaides

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I am indebted to my supervisors Dr Liona Poon and Professor Kypros Nicolaides, for their support and guidance over the last 4 years, without which this work would not have been possible. I am also sincerely grateful to Professor Nicolaides for giving me the opportunity to work and train with him and also experience the magic of his truly unique and inspirational fetal medicine unit.

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I would like to thank my parents for their support and of course, my friend Elina, for always being there. I am enormously grateful to all the women who took part in the screening programme for this work, and feel privileged that their consent allowed me to undertake this research. I would also like to acknowledge a 'write up' grant received from NIHR CLAHRC South London.

# DECLARATION

This thesis entitled ‘Development of a first trimester prediction model for preeclampsia and prospective validation in a multicentre setting’ has been composed by me, Neil O’Gorman, and the work in this thesis is my own. This research project was composed by me with advice from my supervisors Dr Liona Poon and Prof Nicolaides. I worked with the study sponsor, University College London Comprehensive Clinical Trials Unit to keep study documents and site file up to date with the relevant paperwork and substantial amendments whenever necessary.

I was responsible for patient recruitment and acquisition of the biophysical and biochemical markers at King’s College Hospital and North Middlesex University Hospital, as well as liaising closely with fellow colleagues in the remaining participating centres. I played a central role in establishing the infrastructure for the new screening clinics at North Middlesex University Hospital, Lewisham University Hospital and Homerton University Hospital. I also conducted site initiation visits at the participating centres to ensure the adequate training of staff involved in the study.

I contributed to the process of obtaining pregnancy outcomes. I also contributed to ensuring that the outcome measures were accurately recorded in the research database by reviewing obstetric records of women reported as having pre-existing or pregnancy associated hypertension to determine if the condition was chronic hypertension, preeclampsia or gestational hypertension. I wrote and composed this thesis and where information has been derived from other sources, I confirm that this has been indicated in the thesis. I contributed to writing the published papers incorporated in this thesis. This work has not previously been submitted, in part or whole, for consideration for any other degree or professional qualification.

Neil O’Gorman

September 2017

## ABBREVIATIONS

**AUROC:** Area under receiver operating characteristic curves

**ASPRE:** Combined multi-marker screening and randomised patient treatment with ASPirin for evidence-based PREeclampsia

**ACOG:** American Congress of Obstetricians and Gynaecologists

**ART:** assisted reproductive techniques

**β-hCG:** Human chorionic gonadotropin

**BMI:** Body mass index

**BP:** Blood pressure

**cfDNA**: cell free deoxyribonucleic acid

**CH:** Chronic hypertension

**CI:** Confidence interval

**CRL:** Crown-rump length

**CUSUM:** Cumulative sum

**DELFIA:** Dissociation-Enhanced Lanthanide Fluorescent Immunoassay

**DR:** Detection rate

**FPR:** False positive rate

**GA:** gestational age

**GH:** Gestational hypertension

**HLA:** Human leukocyte antigens

**HELLP** haemolytic anaemia, elevated liver enzymes, low platelet count

**ISSHP:** International Society for Study of Hypertension in Pregnancy

**IQR:** Inter quartile range

**IVF:** *In-vitro* fertilisation

**LDL:** low-density lipoproteins

**LMWH:** Low molecular weight heparin

**MAP:** Mean arterial pressure

**MBRRACE-UK:** Mothers and Babies: Reducing Risk through Audits and Confidential Enquiries across the UK

**MoM:** Multiple of median

**NK:** Natural killer

**NT:** Nuchal translucency

**NICE:** National Institute of Health and Clinical Excellence

**OR:** Odds ratio

**PAPP-A:** Pregnancy associated plasma protein-A

**PE:** Preeclampsia

**PI:** Pulsatility index

**PlGF:** Placental growth factor

**PRECOG:** Preeclampsia Community Guideline

**RCT:** Randomized controlled trial

**RR:** Relative risk

**SD:** Standard deviation

**sEng:** Soluble Endoglin

**sFlt-1:** Soluble fms-like tyrosine kinase-1

**SNP:** Single-nucleotide polymorphisms

**TGF-b1**: transforming growth factor-β1

**TV:** transvaginal

**UK:** United Kingdom

**VEGF:** Vascular endothelial growth factor

**WHO:** World Health Organisation

## SUMMARY

Preeclampsia affects 2-3% of all pregnancies and is a major cause of maternal and perinatal morbidity and mortality. In the last ten years, extensive research has been devoted to screening for preeclampsia with the aim of reducing the prevalence of the disease through pharmacologic intervention in the high-risk group and to minimize perinatal morbidity and mortality by tailoring antenatal care accordingly to determine the appropriate time and place for delivery.

The purpose of this study was to develop a first trimester screening model for preeclampsia combining a variety of elements from the maternal demographic characteristics and medical history with biophysical and biochemical markers. This model was subsequently prospectively validated on a new dataset. The efficacy of the developed screening model was then compared to the first trimester approaches to screening recommended by the National Institute for Health and Care Excellence and the American College of Obstetricians and Gynecologists.

The material covered in this thesis was published in three papers in peer reviewed journals. The first publication involved using data from 35,948 singleton pregnancies that included 1,058 preeclamptic pregnancies (2.9%). Bayes theorem was used to combine the *a priori* risk from maternal factors with various combinations of uterine artery pulsatility index, mean arterial pressure, serum pregnancy-associated plasma protein-A, and placental growth factor multiple of the median values. Five-fold cross validation was used to assess the performance of screening for preeclampsia in pregnant women that delivered at <37 weeks' gestation and ≥37 weeks' gestation by models that combined maternal factors with individual biomarkers and their combination with screening by maternal factors alone

In pregnancies that experienced preeclampsia, the values of uterine artery PI and mean arterial pressure were increased, and the values of serum pregnancy-associated plasma protein-A and placental growth factor were decreased. For all biomarkers, the deviation from normal was greater for early than late preeclampsia; therefore, the performance of screening was related inversely to the gestational age at which delivery became necessary for maternal and/or fetal indications. Combined screening by maternal factors, uterine artery PI, mean arterial pressure, and placental growth factor predicted 75%, of preeclampsia <37 weeks and 47% preeclampsia ≥37 weeks' gestation, at a false positive rate of 10%.

The second study examined the diagnostic accuracy of the above model in a prospective first-trimester multicentre study of screening for preeclampsia in 8775 singleton pregnancies. The detection rates (DRs) and false-positive rates (FPRs) for delivery with preeclampsia <32, <37

and ≥37 weeks' gestation were estimated and compared with those for the dataset used for development of the algorithm. In this study population, 239 (2.7%) cases developed PE, of which 17 (0.2%), 59 (0.7%) and 180 (2.1%) developed preeclampsia <32, <37 and ≥37 weeks' gestation, respectively. With combined screening using the above model, the DR was 100% for preeclampsia <32 weeks, 75% for preeclampsia <37 weeks and 43% for PE ≥37 weeks, at a 10% FPR. These DRs were similar to the estimated rates for the dataset used for development of the model: 89% for preeclampsia <32 weeks, 75% for PE <37 weeks and 47% for PE ≥37 weeks.

The third component of this thesis was to compare the performance of screening for preeclampsia based on risk factors from the medical history, as recommended by NICE and ACOG, with our model developed in the first study. Screening with use of NICE guidelines detected 41% of preeclampsia at <32 weeks, 39% of PE at <37 weeks and 34% of PE at ≥37 weeks, at 10.2% FPR. Screening with use of ACOG recommendations detected 94% of PE at <32 weeks, 90% of PE at <37 weeks and 89% ≥37 weeks, at 64.2% FPR. Screening based on the ACOG recommendations for use of aspirin only detected 6% of PE at <32 weeks, 5% of PE at <37 weeks and 2% of PE at ≥37 weeks, at a 0.2% FPR.

The findings of these studies demonstrate that a combination of maternal factors, biophysical and biochemical markers can effectively identify women at high-risk of developing early preeclampsia and that the model developed in this study performs better than the screening approaches recommended by NICE and ACOG.

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# AIMS OF THE THESIS

- To describe the background of preeclampsia, its pathogenesis, the various approaches to first trimester screening for preeclampsia, and potential pharmacological treatment of the disorder.

- To investigate the performance of screening for preeclampsia based on a survival time model which treats gestation at delivery as a continuous variable and using Bayes theorem to combine information from maternal characteristics and biomarker MoM values.

- To prospectively examine, in a European multicentre study, the diagnostic accuracy of the above model for prediction of preeclampsia by a combination of maternal factors and biomarkers at 11-13 weeks' gestation.

- To compare the accuracy of this first-trimester screening model for preeclampsia with that of the models proposed by the American college of Obstetricians and Gynaecologists (ACOG) and the National Institute for Health and Care Excellence (NICE).

# CHAPTER 1 INTRODUCTION

## 1.1 Background

In 1739, Francois Boissier de Sauvages de Lacroix, a French physician described and contrasted an acute form of convulsion with what is now known as epilepsy (Chesley, 1974). He described several species of the genus eclampsia, one of which was *eclampsia parturientum.* It was not until 1840 when proteinuria, in the context of eclamptic seizures, was described and hypertension, recorded by sphygmographic tracings, followed shortly after. The term preeclampsia (PE) was coined in 1894 when it was reported that high blood pressure and proteinuria could occur in pregnant women, without eclamptic seizures (Chesley, 1984).

PE is a multisystem disorder of pregnancy classically characterised with the onset of hypertension after 20 weeks' gestation in the presence of proteinuria. PE typically affects 2-3% of pregnant women (World Health Organisation, 2005; Confidential Enquiry into Maternal and Child Health, 2008; Duley, 2009; Cantwell *et al*., 2011) and it is one of the leading causes of maternal mortality, with an estimated 75,000 maternal deaths worldwide attributed to hypertensive disorders in pregnancy (Khan *et al*., 2006). Severe early PE, requiring delivery <34 weeks' gestation, occurs in 0.3-0.5% of pregnancies making it the most common cause of iatrogenic prematurity and with this brings the associated significant perinatal morbidity and mortality (Witlin *et al.*, 2000; Irgens *et al.*, 2001; von Dadelszen *et al.,* 2003; Papageorghiou, 2008).

PE is a disorder with a wide spectrum. This can range from developing mildly raised blood pressure with proteinuria at term, requiring no further action other than enhanced surveillance and timely induction of labour, to being delivered prematurely due to a fulminating form of the disease potentially leading to multi organ failure and seizures with a peri-viable fetus.

The classic triad of symptoms comprise of headache, visual disturbance and epigastric pain. These are the clinical manifestations of abnormal cerebral perfusion, retinal arteriolar spasm or oedema and hepatic capsular distension, respectively. They are the commonest symptoms that precede an eclamptic seizure. The presence of these symptoms, are not necessarily a prerequisite and at its worst, the disease can even be symptomless.

**Table 1.1.** The four most commonly used definitions of preeclampsia.

| | **WHO 2005** | **NICE 2010** | **ACOG 2013** | **ISSHP 2014** |
|---|---|---|---|---|
| **Systolic/Diastolic** | Systolic<br>Diastolic | Systolic Diastolic | Systolic<br>Diastolic | Systolic Diastolic |
| **Threshold** | > 140/90mmHg on 2 occasions,<br>> 4 hours apart | > 140/90mmHg | > 140/90mmHg<br>on 2 occasions,<br>> 4 hours apart<br>or<br>>160/110mmHg | >140/90mmHg |
| **Baseline** | Normotensive <20 weeks | Normotensive <20 weeks | Normotensive<br><20 weeks | Normotensive <20 weeks |
| **Proteinuria** | >300g/L in 2 random specimens | 300mg/24 hrs<br>PCR >30mg/mmol | 300mg/24 hrs<br>PCR >0.3mg/mg<br>Dipstick + (if above methods not available) | 300mg/24 hrs<br>PCR >0.3mg/mg<br>Dipstick 2+ |
| **Proteinuria necessary for diagnosis** | Yes | Yes | No | No |
| **Other diagnostic clinical criteria** | No | No | Yes | Yes |

WHO - World Health Organization

NICE - National Institute of Health and Clinical Excellence

ACOG – American College of Obstetricians and Gynecologists

ISSHP - International Society for the Study of Hypertension in Pregnancy

## 1.2 Definition of Preeclampsia

PE is broadly defined as development of hypertension and proteinuria in a previously normotensive woman. There are, however, differing opinions as to what the exact diagnostic criteria for the disorder are or should be.

There are several definitions for diagnosis of PE which have been reported in published literature and proposed by various professional bodies Consequently, this has resulted in a number of different guidelines produced by professional bodies worldwide for the diagnosis and management of PE WHO, 2005; NICE, 2010; ACOG, 2013; Tranquilli *et al.,* 2014). A summary of various definitions proposed is shown in Table 1.1. The accepted definition of PE is however, that of the International Society for the Study of Hypertension in Pregnancy (ISSHP) (Davey and MacGillivray, 1988; Brown *et al.,* 2001, Tranquilli *et al.,* 2014).

### 1.2.1 ISSHP Diagnostic criteria

Whether or not to include proteinuria as an essential part of the diagnosis of PE was one of the major reasons for the lack of consensus and controversy regarding diagnosis. This was recognised by the ISSHP and they subsequently recommended that a broad definition, at times not including proteinuria, could be applied for the clinical definition of PE. Stating also that the inclusion of proteinuria, would ensure more specificity around the diagnosis when reporting clinical criteria for patients enrolled in scientific research (Tranquilli *et al.,* 2014).

For the purposes of this research, the ISSHP classification of hypertensive disorders of pregnancy was chosen as it represents a consensus view of an appropriate definition for research use. The ISSHP revised its classification of hypertensive disorders in pregnancy as follows:

**Table 1.2** The revised ISSHP classification (2013) for hypertensive disorders in pregnancy (Tranquili *et al*., 2014).

| The revised ISSHP classification (2013) for hypertensive disorders in pregnancy |
|---|
| • Chronic hypertension<br>• Gestational hypertension<br>• Preeclampsia – *de novo* or superimposed on chronic hypertension<br>• White coat hypertension |

***Hypertension***

Hypertension is diagnosed as a BP of > 140/90 mmHg. The BP should be ideally measured twice by instruments validated for use in pregnancy either automated, liquid-crystal or mercury sphygmomanometer.

***Chronic hypertension (CH)***

Chronic hypertension is hypertension predating the pregnancy. As some women will not have had their BP measured close to pregnancy, in practice the diagnosis of CH is often made in the first trimester.

***Gestational hypertension (GH) and preeclampsia***

New onset of hypertension after 20 weeks' gestation is characteristic of gestational hypertension and PE. The diagnosis of PE is made by hypertension and the coexistence of one or more of the following conditions:

*Proteinuria*

The presence of significant proteinuria defined as presence of more than 300 mg/day protein in a 24 hour urine sample or urine protein/creatinine ratio of > 30 mg/mmol.

*Maternal organ dysfunction*

This is characterised by:

- Renal insufficiency: characterised by serum creatinine levels of > 90 μmol/L or 1.02 mg/dL
- Liver involvement: characterised by elevated transaminases with levels at least twice the upper limit of normal range with or without right upper quadrant or epigastric abdominal pain
- Neurological complications: characterised by hyperreflexia accompanied by clonus; severe headache accompanied by persistent visual scotoma; blindness, stroke or eclampsia
- Haematological complications: characterised by platelet count below 150,000/dl

*Utero-placental dysfunction*

This is characterised by presence of fetal growth restriction.

Gestational hypertension (GH) is diagnosed when hypertension develops *de novo* after 20 weeks' gestation and is not accompanied by any of the above features. PE superimposed on CH is diagnosed when the hypertension is accompanied by one or more of the above features.

***White coat hypertension***

This is hypertension uniquely diagnosed in clinical setting with normal BP measurements obtained in the home setting. This diagnosis can be confirmed by carrying out 24 hour ambulatory BP monitoring.

***Gestational proteinuria***

This is diagnosed as a finding of significant proteinuria as per the above definitions without the accompanying hypertension or primary renal disease.

**1.2.2 Classification of PE**

There remains much debate on how the disorder should be classified. Some classifications define PE as pregnancy induced hypertension in association with evidence of multi-organ dysfunction; proteinuria, or other complications such as renal insufficiency, liver disease, neurological problems, haematological disturbance, or fetal growth restriction (North *et al.,*

2011). Other authors have distinguished between proteinuric and non-proteinuric PE, reporting that the former carries a worse prognosis than non-proteinuric PE, which is in turn thought to carry a worse prognosis than gestational hypertension alone (Homer *et al.,* 2008).

**Table 1.3** The revised ISSHP definition of preeclampsia (Tranquili *et al*., 2014).

| Hypertension developing after 20 weeks co-existing with new onset of one or more of the following features |
|---|
| • Proteinuria<br>• Maternal organ dysfunction<br>- Renal insufficiency<br>- Liver involvement<br>- Neurological complications<br>- Haematological complications<br>• Utero-placental insufficiency<br>- Fetal growth restriction |

Opinions differ widely over whether early and late variants of PE are the same disease, or whether they have a completely different pathophysiology to each other. In general, the maternal risk factor profiles vary between the two variants. An alternative hypothesis is that PE is a spectrum disorder, and the more severe the disease is, the earlier the gestation at which the delivery will be. A novel approach for screening for PE involving multiple maternal characteristic variables and biomarkers in which, the gestation at the time of delivery for PE is treated as a continuous rather than a categorical variable. This method, based on a survival time model, allows estimation of individual patient-specific risks of PE requiring delivery before a specified gestation. This approach considers a situation that if a pregnancy continued indefinitely, all women would develop PE. Whether they develop PE or not depends on the competition between delivery before or after development of PE (Wright *et al.,* 2012). This theory has contributed significantly to the most successful approach to preeclampsia prediction and will be discussed further in the subsequent chapters.

## 1.3 Pathogenesis of Preeclampsia

Although the exact cause of PE remains elusive, the condition is thought to be predominantly due to defective implantation of the placenta within the uterine endometrium. The pathogenesis is hypothesised to occur in two main phases. The first phase begins with abnormal placentation, while the second phase is characterised by an abnormal maternal endothelial response, resulting in the clinical manifestations of the condition including hypertension, proteinuria and oedema. Figure 1.3 summarises the pathogenesis of PE.

### 1.3.1 Placental phase

*Normal Placentation of Pregnancy*

The uterus is supplied by the two uterine arteries, with some collateral circulation from the ovarian arteries. The circumferential delivery of blood to the uterus is achieved via the arcuate arteries that arise from the uterine arteries (Figure 1.1). These vessels penetrate the myometrium of the uterus in the form of radial arteries and give rise to

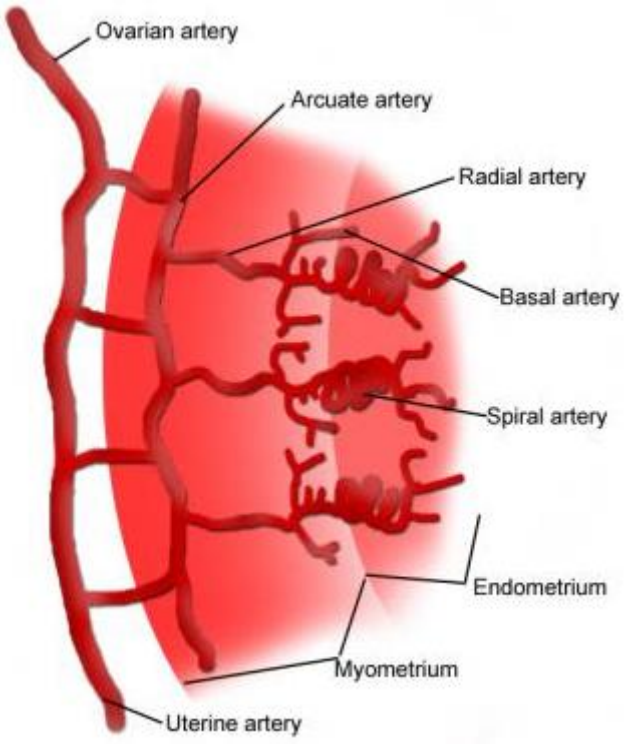


**Figure 1.1** The blood supply to the uterus (taken from http://emedicine.medscape.com)

the basal and spiral arteries, which supply oxygenated blood to the myometrium, decidua and the intervillous spaces in the placenta during the pregnancy. The placenta develops primarily from fetal derived trophoblastic cells. The trophoblast is the first cell linage to differentiate at

the stage of the blastocyst, occurring approximately 6 days post conception (Huppertz and Herrler, 2005). Upon blastocystic implantation, these trophoblastic stem cells undergo mitosis and differentiate into two types: the cytotrophoblasts, which are the precursors to all subsequent trophoblast cells and at the attachment site, the syncytiotrophoblasts; those responsible for the invasion into the decidua, and in particular, the maternal spiral arteries. Under the influence of certain growth factors, including vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), a definitive blood supply is established with both the formation of new vessels (vasculogenesis) and branching from existing vessels (angiogenesis). Approximately 4-6 weeks post-conception, the cytotrophoblasts differentiate into invasive extravillous cytotrophoblasts and disrupt the maternal vascular integrity by migrating into the decidua and invading the maternal spiral arteries – a process known as endovascular invasion. Interstitial invasion also occurs, in which these differentiated cells bury through into the myometrium underlying the placental bed. Initially, these interstitial cytotrophoblasts remain adjacent to the spiral arteries, but from 8 weeks of gestation onwards, invasion of the inner third of the myometrium occurs and the cytotrophoblasts become more broadly distributed (Pijnenborg *et al.*, 1991). The fusion of these interstitial cytotrophoblasts with the formation of multi-nucleated giant cells is thought to influence the prevention of further myometrial invasion (Pijnenborg *et al.*, 1983).

Endovascular trophoblasts are found within the lumen of the spiral arteries from 4 weeks of gestation and they migrate towards the proximal part of the vessel (Pijnenborg *et al.*, 1991). They line the endothelium and disrupt the walls of the intra decidual portion of the spiral vessels, thus destroying the muscular and elastic integrity of the media. The vessel wall is eventually replaced by a fibrinoid material originating from both, fibrin in the maternal blood and proteins secreted by the trophoblast. These small-diameter high resistance spiral arteries become grossly dilated, funnel-shaped, flaccid utero placental arteries with diameters increasing from 15-20 to 300-500 μm. The high resistance in the utero placental circulation is transformed to a circulation of low resistance and higher volume than that which is present in the pre-pregnancy state. This fall in vascular resistance is facilitated by the loss of normal vasomotor control of the spiral arteries as the muscular and neural layers are no longer intact. This allows the required ten-fold increase in uterine blood flow to meet the nutritional needs of the developing fetus. Crucially, this physiological process is thought to occur in two stages: the initial wave of trophoblastic invasion, from approximately 8 weeks of gestation, involves the decidual portion of the spiral arteries. The second wave occurs at the junctional zone of the spiral arteries at the level of the myometrial segments and occurs at 14-24 weeks of gestation (Lyall, 2002).

*Placentation in Preeclampsia*

In PE, the physiological processes of vasculogenesis and angiogenesis described, as well as the transformations of the spiral arteries, do not occur as they should. There is inadequate invasion of the placenta by the cytotrophoblastic cells at the levels of both the decidua and the myometrium (Khong *et al.*, 1986). Even though 50-70% of the spiral arteries display some form of invasion, this invasion is confined to the decidual part of the vessels. It is the second wave of invasion that appears to be defective, as the myometrial segments remain significantly unphased (Brosens *et al.*, 1972; Gerretsen *et al.*, 1981; Meekins *et al.*, 1994a).

Atherosclerosis is also a common histological feature found in placentas of pregnancies complicated by PE (Sheppard and Bonnar, 1981). Macrophagic infiltration of the vessel walls causes a fibrinoid necrosis and a perivascular mononuclear cellular infiltrate (Pijnenborg *et al.*, 1991). The walls of the vessels of the utero placental circulation are subjected to a higher density of lipoproteins (Meekins *et al.*, 1994b) and within these vessels, micro thrombi contribute to the macroscopic infarction of the placenta (Salafia *et al.*, 1995).

This acute atherosis is not unique to PE. It is also observed in the placentas of pregnancies affected by fetal growth restriction, antiphospholipid syndrome and spontaneous miscarriage. The former, with or without PE, can be associated with the failure of trophoblastic invasion of the maternal spiral arteries (Frusca *et al.*, 1989).

Research has shown that high resistance in the Doppler studies of uterine arteries, in pregnancies affected by PE, correlates well with histological findings from placental bed biopsies of such pregnancies (Olofsson *et al.*, 1993; Meekins *et al.*, 1994a; Sagol *et al.*, 1999).

Not all cases of PE are associated with impaired placentation (Pijnenborg *et al.,* 1991; Meekins *et al.*, 1994a), however, a number of studies suggest that the gestational time of onset is related to the degree of the placental histological insult (Moldenhauer *et al.*, 2003; Egbor *et al.*, 2006). In late PE, the placental morphology and histology are more likely to resemble those of normotensive pregnancies. It has been shown that a diagnosis of PE is more likely to be made prior to 32 weeks of gestation in those with progressive utero placental circulation compromise (Ghidini *et al.,* 1997).

There is considerable overlap in the degree of placental vascular lesions between normal and PE pregnancies (Ghidini *et al.*, 1997) and similarly, a degree of utero placental vascular pathology does not render a pregnancy destined to yield significant morbidity and/or mortality (Aardema *et al.*, 2001). This has been corroborated by studies that have shown that trophoblastic invasion into the myometrial segments can still occur in PE (Sheppard and

Bonnar, 1981). Pijnenborg *et al.* (1991) showed that trophoblastic invasion of a vessel wall can vary in depth from affecting only a segment of the arterial wall to the whole circumference of the artery. However, the severity of changes within the spiral arteries does not necessarily correlate with the clinical manifestations of the disorder.

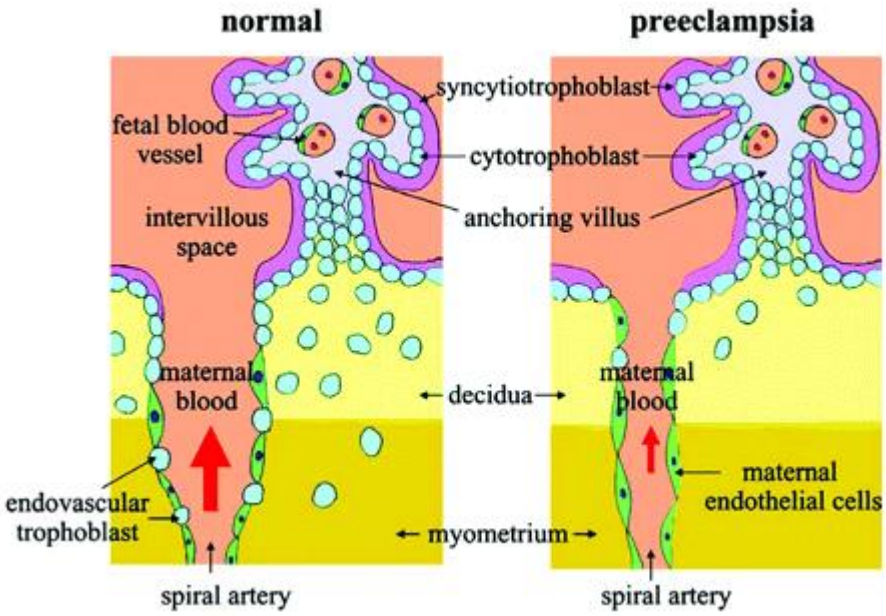


**Figure 1.2** Normal and abnormal placentation (taken from Kita and Mitsushita, 2008)

In summary, there can be a significant disruption to the normal physiological placentation process that can diminish the quality and quantity of maternal revascularisation in pregnancies complicated by PE (Figure 1.2). The consequent high-pressure system restricts the necessary physiological increase in blood supply required by the developing fetus and poor utero placental perfusion ensues. Normal placentation may, however, be found in pregnancies affected by PE, thus the correlation between abnormal placentation and subsequent adverse pregnancy outcome is not exact. The insufficient trophoblastic invasion should be considered, therefore, more of a predisposing factor, with the maternal response syndrome contributing to the clinical signs of the disorder.

### 1.3.2 Genetic factors

A genetic predisposition to PE may be a factor for those that develop the disorder. Women with a positive family history have an estimated threefold risk for developing the condition

compared to those without a family history of PE (Arngrimsson *et al*., 1990; Duckitt and Harrington, 2005).

The presence of such 'preeclampsia susceptibility genes' or a combination of these genes or their alleles may increase the risk of PE by altering the regulation of blood pressure and/or by influencing and modifying placental function (Cooper and Liston, 1979; Chesley and Cooper, 1986). The proposed genetic models are either a single recessive gene or a single dominant gene (Cooper and Liston, 1979; Chesley and Cooper, 1986). There have been reported associations between PE and polymorphisms of various genes such as angiotensinogen, tumour necrosis factor-a (TNF-$\alpha$), factor V Leiden and the 5,10-methylenetetrahydrofolate reductase genes (Morgan *et al.,* 1999; Lachmeijer *et al.,* 2001; Benedetto *et al.,* 2002; Heiskanen *et al.,* 2002).

In a recent systematic review of literature and meta-analysis (Human Genome Epidemiology Review [HuGE review]), the authors examined the association between maternal genotype and severe PE according to certain gene groups including immune, cell signalling, metabolic, thrombophilic and vasoactive groups. They analysed 57 studies which evaluated 50 genotypes in 5,049 cases and 16,989 controls and found that a high risk for severe PE was noted with coagulation factor V gene (proaccelerin, labile factor) polymorphism rs6025, coagulation factor II (thrombin) gene mutation G20210A, leptin receptor gene (LEPR) polymorphism rs1137100 and the thrombophilic gene group (Fong *et al.,* 2014).

In another meta-analysis, the authors specifically examined the relationship between the risk of PE and two thrombophilia single-nucleotide polymorphisms (SNPs), the factor V G1691A SNP and prothrombin G20210A SNP. They examined 37 studies with 5,048 patients with PE and 6,796 controls and reported that both these polymorphisms are associated with increased risk of all types of PE, including severe PE (Wang *et al*., 2014). In this publication, the presence of a prothrombin 20210A polymorphism doubled a patient's risk of developing PE and an approximate 3-fold increase in risk for severe PE. The authors discuss that although associations between gene polymorphisms and PE has been illustrated, to explore this further and establish a causal relationship, there is a need for larger prospective studies.

### 1.3.3. Immunological factors

As the placenta originates from fetal cells, there are both maternal and paternal derived antigens. In pregnancies unaffected by PE it appears that the maternal immune system tolerates the fetal antigens and mounts a modified immune response to allow acceptance of the semi-allogenic fetus. To facilitate this, there must be a change within immune pathways with an intact innate immune system.

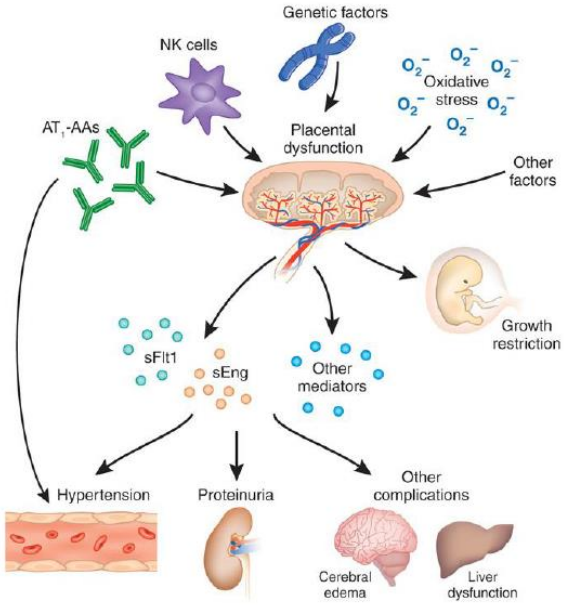


**Figure 1.3** A summary of the pathogenesis of PE (Parikh and Karumanchi, 2008).

Natural Killer (NK) cells, macrophages and dendritic cells are mediators of the innate immune system. NK cells have been shown to initiate smooth muscle remodelling of the maternal spiral arteries (Robson *et al.*, 2011). Macrophages and dendritic cells act as major antigen presenting cells within the uterus. The presence of these cells permits the maternal immune system to adapt and thus prevent pregnancy loss. Studies of placental macrophages suggest that in pregnancies affected by PE, there are significantly more macrophages in this cohort's placentas, and that macrophage infiltration is implicit in impaired trophoblast invasion (Zhang *et al.*, 2013).

It has been shown that in pregnancies with PE, an immunological cascade is activated producing a reaction to both the fetus and the placenta and particularly against the paternal portion of the trophoblasts (Dekker *et al*., 1998; Dekker and Robillard, 2005). It remains unanswered whether this immune reaction is a consequence of the abnormal placentation or if it is in fact, an aetiological factor (Djurisic and Hviid, 2014).

There are two classes of human leukocyte antigens (HLA): class I and II. The class HLA Ia antigens (A, B and C) and HLA II (DR, DQ and DP) are concerned with antigen presentation and therefore in organ transplantation and autoimmunity. The HLA Ib (E, F and G) genes are primarily expressed in the extravillous trophoblast cells lining the placenta and by syncytiotrophoblast cells (Ishitani *et al.,* 2003; Bhalla *et al., 2*006) and have drawn the attention of researchers for this reason. Uniquely, the HLA-G is expressed on the surface of the trophoblast. A possible altered maternal immune response against the paternally-derived

antigens on the trophoblast is hypothesised as a potential aetiological factor. Sargent *et al.* (2006) proposed that PE is a syndrome in which the first stage, the inadequate trophoblastic invasion leads to depressed expression of HLA-G causing a diminished stimulation of decidual NK cells that are essential for the production of immunoregulatory cytokines and angiogenic factors. The second stage involves leukocytes and the endothelium in a systemic inflammatory response type syndrome. This inflammatory stimulus is thought to originate in the placenta and may be caused by the release of necrotic and/or apoptotic syncytiotrophoblasts into the maternal circulation.

Epidemiological data supports these hypotheses as PE is more common in a first pregnancy, the risk for PE decreases with subsequent pregnancies (with the same father), changing partners results in a loss of the parity protection against PE (Robillard *et al.,* 1999; Li and Wi, 2000) and lastly, donor insemination techniques and oocyte donation both increase the likelihood for the development of the condition (Smith *et al.,* 1997; Wilson *et al.,* 2003).

### 1.3.4. Inflammation and oxidative stress

Normal pregnancies are associated with a maternal systemic inflammatory response, and this response is exaggerated in preeclamptic pregnancies (Redman *et al.*, 2003). This inflammatory state activates several associated pathways, including those involved in oxidative stress. One of which is the production of reactive oxygen species (ROS) leading to oxidative stress when the inherent anti-oxidant mechanisms in tissues are overwhelmed. In pregnancies with PE, the impairment in placentation leads to the sequence of placental hypoxia and re-oxygenation causing an increased production of ROS, which in turn contributes to the oxidative stress (Burton and Jauniaux, 2011). This oxidative stress has been shown to be involved in vasculopathy and has therefore been postulated to be involved in preeclampsia development. Some work has evaluated the link with prevention of preeclampsia with anti-oxidants such as vitamins C and E. This is described in detail in section 1.5.3. In summary, placental hypoxia leads to oxidative stress causing a release of proinflammatory cytokines and trophoblast debris into the circulation, contributing to the pathophysiology of PE (Cindrova-Davies *et al.,* 2007).

Placental oxidative stress resulting from the ischaemia–reperfusion injury is involved in the pathogenesis of PE (Gupta *et al*., 2005). Placental lipid peroxidation levels are significantly higher in the placentas of PE (Walsh *et al.*, 2000; Atamer *et al.*, 2005, Vanderlelie *et al.*, 2005). Dyslipidaemia early in pregnancies complicated by PE can lead to the accumulation of very low-density lipoproteins (VLDL) in the subendothelial space. These are then oxidised to form highly reactive oxidised LDL which disrupt the membrane proteins and phospholipids and increase the expression of signalling molecules which recruit inflammatory cells. The injured

membrane alters endothelial function while monocytes take up oxidised LDL to form foam cells and eventually the fatty streak characteristic of atherosclerosis (Roberts and Cooper, 2001). In PE, the reduced reserve in net anti-oxidant activity exacerbates this damage caused by these oxygen free radicals.

### 1.3.5. Endothelial dysfunction

Although multiple aetiologies have been attributed to causing PE, it is placental hypoperfusion leading to ischaemia and necrosis/infarction that is thought to be the end point that results in the clinical manifestations of this syndrome. Endothelial cell activation is hypothesised to be a prominent pathophysiological feature of PE. This is underlined by the increased vascular resistance, which is also key feature of the disorder. It has been well documented that impaired placentation and the resulting placental hypoxia can lead to intravascular inflammation and consequent activation or repression of endothelial cell function (Redman, 1991; Gervasi *et al.,* 2001; Redman and Sargent, 2003; Myatt and Webster, 2009; Saito and Nakashima, 2014).

The consequent endothelial dysfunction alters the equilibrium of angiogenic and anti-angiogenic proteins. The altered concentration of these proteins further exacerbates the endothelial dysfunction established by intravascular inflammation (Luttun *et al.,* 2002; Maynard *et al.,* 2003; López-Novoa, 2007; Widmer *et al.,* 2007; Zhou *et al.,* 2011; Murphy *et al.,* 2013). The damaged placental tissue releases receptor proteins such as soluble fms-like tyrosine kinase (sFlt-1), which is a protein produced by the syncytiotrophoblast and is formed by alternative splicing of the Flt-1 gene. It antagonises the actions of proangiogenic growth factors, such as VEGF and PlGF (Kendall and Thomas, 1993; Huppertz *et al.*, 2008), by attaching to the transmembrane receptors, thus preventing these proangiogenic factors from interacting with their receptors (Maynard *et al.,* 2003; Levine *et al.,* 2004; Romero *et al.,* 2008; Taché *et al.,* 2011).

VEGF is a protein released by many cell types including the cytotrophoblast and it is involved in angiogenesis and vasculogenesis. It is transcribed from the VEGF gene, which is located on chromosome 6, which also encodes for various isoforms of VEGF including PlGF (Romero *et al.,* 2008; Cheng *et al.,* 2013). These angiogenic factors are crucial in mediating increased vascular permeability, which includes angiogenesis, vasculogenesis and growth of endothelial cells (Yamazaki *et al.,* 2006; Maharaj *et al.,* 2008). VEGF is vital in the preservation of endothelial function in fenestrated endothelium, particularly in the brain, liver and renal glomeruli (Esser *et al.,* 1998). Higher levels of sFlt-1 counteract vasodilatory effects of nitric oxide, which is induced by VEGF, resulting in maternal hypertension (Maynard *et al.,* 2003).

Soluble endoglin (sEng) is another anti-angiogenic factor implicated in the pathogenesis of PE. It is a surface receptor of transforming growth factor-β1 (TGF-β1), which induces growth and proliferation of endothelial cells (Venkatesha *et al.,* 2006). Its anti-angiogenic properties are achieved by preventing the binding of TGF-β1 to its receptors on endothelial cells thereby, compromising their function and leading to impairment in production of nitric oxide (Levine *et al.,* 2006). sEng amplifies vascular damage mediated by sFlt-1 and the two together synergistically, can lead to severe vascular damage, nephrotic syndrome, proteinuria and fetal growth restriction (Venkatesha *et al.,* 2006). Studies have shown that when sEng is administered to rodents, it induces a severe preeclampsia-like disorder (Maynard *et al*., 2011). It is the imbalance of these critical angiogenic proteins that cause systemic vascular dysfunction, capillary leaking and vasospasm, leading to the clinical spectrum of preeclampsia.

Endothelial cell dysfunction affects vascular tone vasospasm and platelet activation and Romero *et al.* (1988) showed that even prior to the onset of hypertension, platelet dysfunction and thrombocytopaenia can be present. There are also alterations in the levels of prostacyclin and thromboxane A2. Prostacyclin is a vasodilator and inhibits platelet aggregation in contrast to thromboxane A2, which constricts blood vessels and promotes platelet aggregation (Romero *et al.,* 1988). Prostacyclin concentration in maternal blood and urine in pregnancies with PE is reduced (Bussolino *et al.,* 1980) and as expected, there is an increased production of thromboxane A2 with this disorder (Walsh, 1985). Consequently, there is increased platelet activation, resulting in platelet aggregation and formation of thrombi in the microcirculation of different organs, which is a major contributor to pathogenesis of PE (Romero and Duffy, 1980).

## 1.4 Prediction of Preeclampsia

### 1.4.1 Background

PE and eclampsia are major causes of maternal mortality in the UK (Knight *et al (Eds).,* on behalf of MBRRACE-UK, 2016) although there has been a significant reduction in maternal mortality rates over the last 50 years. The traditional method for early detection and diagnosis of PE is to serially measure blood pressure and assess the urine for protein at regular intervals at antenatal clinics. For women with no predisposing risk factors to PE, the Preeclampsia Community Guideline (PRECOG) (Milne *et* al., 2005) recommends following the National Institute for Health and Care Excellence (NICE) antenatal care for uncomplicated pregnancy guidelines (2008). However, if they have one or more risk factors (Table 1.4), they should be reviewed by a healthcare professional at least every 3 weeks between 24 to 32 weeks' gestation and a minimum of every two weeks from 32 weeks' gestation to delivery (Milne *et*

al., 2005). This approach is not useful for predicting or identifying high-risk women that are likely to go on to develop PE. Although recognition of maternal risk factors is useful in clinical practice, it cannot be used reliably to screen and predict PE (Wallenburg, 2001). In a statement published in 2017, which reaffirms the conclusions of their published guidance on the management of hypertensive disorders in pregnancy (ACOG, 2013), ACOG advocates there are no accurate, predictive tests at this time to determine whether a woman will develop preeclampsia and therefore continues to recommend against other methods for predicting PE. This document states that a detailed medical history and routine blood pressure measurements are the best tools available to alert a healthcare professional of a potential risk.

**Table 1.4** PRECOG recommendations of risk factors for PE

| Risk factor |
| --- |
| First pregnancy |
| Multiparous with<br>• PE in any previous pregnancy<br>• Ten years or more since last delivery |
| Age 40 years or over |
| Body Mass Index of 35 or more |
| Family history of PE (mother or sister) |
| Booking diastolic BP >80mmHg |
| Booking proteinuria (of ≥ 1+ on more than one occasion or quantified at ≥ 0.3g/ 24 hr) |
| Multiple pregnancy |
| Certain underlying medical conditions<br>• Pre existing hypertension<br>• Pre existing diabetes<br>• Pre existing renal disease<br>• Antiphospholipid antibodies |

In addition, NICE (2008 and 2010) published guidelines for antenatal care in the UK and recommended that a woman's level of risk for PE, based on her demographics and factors in her medical history, should be determined at the initial booking appointment and her subsequent intensity of antenatal care should be tailored according to this assessment of risk (Table 1.5). According to ACOG (2015), the risk factors for PE are nulliparity, age> 40 years, BMI ≥30 kg/$m^2$, conception by *in-vitro* fertilization, history of previous pregnancy with PE, family history of PE, chronic hypertension, chronic renal disease, diabetes mellitus, systemic lupus erythematosus or thrombophilia. The ACOG (2013) had previously specified their indications for the initiation of aspirin prophylaxis (Table 1.5). No available data exists on the performance of such a recommended screening strategy which treats each risk factor as separate screening tests. Such an approach to the screening of PE is likely to result in falsely classifying a large

number of pregnant women as screen-positive who will need more frequent antenatal monitoring, thereby undermining the very purpose of screening. In turn, it also creates a substantial and possibly avoidable strain on the healthcare system.

**Table 1.5** NICE & ACOG recommendations for managing patients at high risk of preeclampsia

| NICE 2008* | NICE 2010/11^ | ACOG 2013± |
|---|---|---|
| - Age ≥40 years<br>- Nulliparity<br>- Pregnancy interval of more than 10 years<br>- Family history of PE<br>- Previous history of PE<br>- Body Mass Index of 30 Kg/m2 or above<br>- Pre-existing vascular disease such as hypertension<br>- Pre-existing renal disease<br>- Multiple pregnancy | **High Risk Factors**<br>- Hypertensive disease during a previous pregnancy<br>- Chronic kidney disease<br>- Autoimmune disease (SLE/APS)<br>- Pre-existing diabetes<br>- Chronic hypertension.<br><br>**Moderate Risk Factors**<br>- First pregnancy<br>- Age >40 years<br>- Pregnancy interval >10 years<br>- BMI >35kg/$m^2$ at 1$^{st}$ visit<br>- Family history of PE | - History of PE in two or more previous pregnancies<br>or<br>- PE requiring delivery<34 weeks' gestation |

* More frequent blood pressure measurements should be considered for pregnant women with above risk factors

^ Aspirin 75mg daily until delivery

± Aspirin 60-80mg daily

SLE = Systemic Lupus Erythematosus, APS = Antiphospholipid syndrome

### 1.4.2 Maternal demographic factors and obstetric history

*Maternal age*

There is no shortage of studies published that have suggested there is an increase in the risk of PE with advancing maternal age. Previous studies, which used maternal age with cut-offs at either 35 or 40 years of age, have shown a 2-fold increase in the risk of developing PE (Bianco *et al.,* 1996; Lawoyin and Ani, 1996; Lee *et al.,* 2000; Chen *et al.,* 2000). The risk for PE also appears to increase more abruptly after the mid-30s in another study (Saftlas *et al.,* 1990) and similarly reported in a study by Mittendorf *et al.,* (1996) the risk for PE increases by 30-40% for every additional year past the age of 34 years.

These findings were further corroborated in a study which confirmed the association of advanced maternal age with PE and this risk of PE was significantly higher in mothers older than 40 years compared to younger mothers. This association remained statistically significant regardless of adjustment for parity (Ziadeh and Yahaya, 2001). Another study that reported this association also examined the risk factors for PE in a multivariate approach, thus accounting for confounding effects and interactions, and reported that the risk for late onset PE increases by 4% every year over the age of 32 years (Poon *et al.,* 2010). Advancing age is not only associated with PE but also their risk of developing early PE is higher than developing late PE (Khalil *et al.,*2013a).

*Parity & Change of Partner*

In primigravidas, the elevated risk of developing PE is reported widely. In fact, it is reported to increase this likelihood 3-fold (Duckitt and Harrington, 2005). In a systematic review of 26 studies, researchers found that this elevated risk for PE remained even after adjusting for other risk factors such as maternal age, race and body mass index and the summary adjusted odds ratio was 2.71 (Luo *et al.,* 2007). Interestingly, women who have previously conceived, and even after a miscarriage, appear to have some degree of protection against PE (Dekker *et al.,* 1998). This can be explained by the immune maladaptation hypothesis which states that the fetal-placental unit contains paternal antigens that provoke an abnormal maternal immune response in PE (Dekker, 1999). This hypothesis is strengthened as multiparity appears to reduce the risk of PE and with a change of partner, this protective effect is lost (Robillard *et al.,* 1993). It is thought that regular sperm exposure over a prolonged period prior to conceiving may prime the mother's immune system and therefore creating a protective effect against PE in nulliparous mothers (Marti and Herrmann, 1977; Dekker 2002; Dekker and Robillard, 2005).

*Inter-pregnancy interval*

It is also possible that the increased risk of PE associated with a change of partner may in fact be, attributable to a longer inter-pregnancy interval. The importance of the inter-pregnancy interval comes from a cross-sectional study which reported a significant increase in the risk of PE in women with inter-pregnancy intervals of more than 59 months compared with those with intervals of 18-23 months (Conde-Agudelo *et al.,* 2000). A large Norwegian population study by Skjaerven *et al.* (2002) examining 550,000 women who had two or more singleton deliveries and 200,000 women who had three or more singleton deliveries demonstrated an association between the risk of PE and the inter-pregnancy interval is more significant than the association between the risk and a change of partner. More specifically, the risk in a second or third pregnancy is directly related to the inter-pregnancy interval. When this interval is 10 years or more, the risk of developing PE is similar to that of a nulliparous woman. After adjusting for the possibility of a change of partner, maternal age and year of delivery, the probability of PE is increased by a factor of 1.12 for each year increase in the inter-pregnancy interval.

*Parous women with history of previous preeclampsia*

Results from observational studies suggest that having had PE increases the risk of recurrence in subsequent pregnancies. This risk has been quoted to be between seven to ten times higher in a second pregnancy (Campbell *et al.,* 1985; Sibai *et al.,* 1986; Lie *et al.,* 1998; Lee *et al.,* 2000; Mostello *et al.,* 2002; Duckitt and Harrington, 2005). Inversely, women with PE in their second pregnancy are seven times more likely to have suffered from PE in their previous pregnancy compared to women in their second pregnancy who did not develop PE (Eskenazi *et al.,* 1991; Stone *et al.,* 1994).

Quantifying such recurrence risks has proven more difficult. In a meta-analysis that examined the recurrence of hypertensive disorders in pregnancy, the authors examined data from 94 studies including more than 99,000 pregnancies. They found the recurrence rates for hypertensive disorders in pregnancy, PE and GH were 20.7%, 13.8% and 8.6%, respectively. This study also found an inverse relationship between the gestational age at delivery in the first pregnancy and the risk of PE in a subsequent pregnancy (van Oostwaard *et al.,* 2015).

A history of PE doubles the risk of developing early PE (<32 weeks) in a subsequent pregnancy as opposed to late PE (Odegard *et al.,* 2000). There are other studies reporting their results on the recurrence risk for a subsequent early PE (<34 weeks) for those a previous history of early PE in the index pregnancy. This risk ranges from 5% to 17% (van Rijn *et al.,* 2006; Langenveld *et al.,* 2010). In a systematic review examining the risks of early delivery at <34 weeks following early PE in the first pregnancy, the authors reported results from 11 studies

including 2,377 women and found that the pooled recurrence risk for early disease is approximately 8% (Langenveld *et al.,* 2011).

*Assisted reproductive technologies*

There are multiple studies showing that assisted reproductive techniques (ART) double the risk for PE (Maman *et al.*, 1998; Jackson *et al.*, 2004; Shevell *et al.*, 2005; Lambert-Messerlian *et al.*, 2006; Trogstad *et al.*, 2009). Whether *in vitro* fertilisation (IVF) and simple ovulation induction inflict the same susceptibility to the disorder, remains unclear. The evidence is conflicting as one large observational study has shown that it is IVF that increases the risk for PE and not ovulation induction (Shevell *et al.*, 2005) and another case-control study has found that both techniques are equally as likely to contribute to developing hypertension in pregnancy (Maman *et al.*, 1998). On the contrary, Trogstad *et al.* (2009) has reported that it is ovulation induction rather than IVF that increases the risk of PE by two-fold.

A cohort study of 47,088 pregnancies following ART showed that the risk of PE was higher in IVF pregnancies compared to pregnancies conceived spontaneously and, the risk was even higher in frozen-thawed cycles compare to fresh cycle pregnancies (Opdahl *et al.,* 2015). Those who undergo donor IVF appear to have a higher risk for PE than those women having autologous ovum IVF (Simeone *et al.,* 2014). There is also some evidence from IVF pregnancies with ovum donation that there is an altered extravillous trophoblast and immunological changes in decidua basalis which may impede the modification of the spiral arteries (Nakabayashi *et al.,* 2015). A systematic review of 19 studies including more than 86,000 pregnancies supported these findings by showing that the risk of PE is higher in oocyte donation IVF cycles compared to the other methods of ART and natural conception (Masoudian *et al.,* 2015).

*Racial origin*

Extensive evidence in the literature clearly shows the association between certain racial origins and PE. A small series performed by Eskenazi *et al.* (1991) demonstrated that Afro-Caribbean race is associated with a 12-fold increase in the risk of PE but numerous larger studies suggest this number to be closer to a 20-50% increase in the risk (Mittendorf *et al.,* 1996; Sibai *et al.,* 1997; Knuist *et al.,*1998; Mostello *et al.,*2002; Caughey *et al.,* 2005). The risk for PE is also higher in women of South Asian origin. These differences in risks for PE also mirror the different metabolic profile of non-pregnant women, who have an increased susceptibility to cardiovascular disease. Both Afro-Caribbean and South Asian women are more susceptible to developing chronic hypertension and cardiovascular disease. Afro-Caribbean women are more likely to suffer a stroke and renal failure whereas South Asian women are more at high-risk of

coronary heart disease (Cappuccio 1997a; Cappuccio *et al.,* 1997b; Ramaraj and Chellapa 2008).

In contrast, women of East Asian origin have a significantly lower risk of PE compared to Caucasian women. A retrospective cohort study including 67,746 pregnancies examining the risk of developing PE reported that in East Asian women of Chinese descent have a significantly lower risk compared to Caucasian women and the possible explanations for this difference could be attributed to the difference in BMI and lifestyle factors such as length of cohabitation with partner (Xiao *et al.,* 2014).

In a large prospective observational cohort study of more than 79,000 singleton pregnancies that looked at the association of maternal race with pregnancy outcomes, reported that the risk of PE was significantly higher in women of Afro-Caribbean and South Asian racial origin compared to Caucasian women. This increase in risk remained significant even after adjusting for other confounding factors. In fact, after chronic hypertension, Afro-Caribbean race was the second highest risk factor associated with risk for developing PE with an OR of 2.60 (Khalil *et al.,* 2013b).

*Cigarette smoking*

There is little doubt that cigarette smoking has an adverse effect on pregnancy. It has a known association with chronic lung disease, preterm delivery and placental abruption, and it is also associated with an increased risk of fetal complications such as stillbirth, fetal growth restriction (FGR), placenta praevia and spontaneous miscarriage.

However, there is evidence in the literature suggesting that the risk of PE is reduced in women who smoke cigarettes during pregnancy. A systematic review of 28 cohort and 7 case-control studies that included over 800,000 women, reported that cigarette smoking in pregnancy reduces the overall risk of PE by 30% (Conde-Agudelo *et al.*, 1999). In an inverse dose-response relationship manner, the risk of PE declined as the number of cigarettes smoked daily during the pregnancy increased. A meta-analysis of 9 cohort studies showed that smokers of less than 10 cigarettes per day and 10 or more cigarettes per day had 20% and 30% reductions, respectively, in the risk of PE. Similar findings were observed with data pooled from 3 case-control studies (10% and 40%, respectively) (Conde-Agudelo *et al.*, 1999).

The GOPEC (Genetics of Pre-Eclampsia) consortium analysed over 1000 women with moderate to severe preeclampsia in a multi-centre study, and carried out a sub-analysis to analyse the relationship between smoking and PE. They found the risk of developing eclamptic seizures was increased five-fold in smokers compared to non-smokers, suggesting that

although smokers are less likely to develop PE, but if they do, they develop a more severe form of the disorder (Pipkin FB, Genetics Preeclampsia Consortium, 2008).

Another interesting observation documented from a Swedish population study was that a change in smoking habits during pregnancy can affect the risk for PE. Women who reported smoking at the first antenatal visit but stopped subsequently, did not have reduced rates of PE. However, those who reported no smoking initially, but were smoking at 30-32 weeks' gestation had reduced rates of PE, suggesting that smoking in the second half of pregnancy has a protective effect of the development of PE (Wikstrom *et al.,* 2010).

*Family history of preeclampsia*

The risk for PE appears to be increased 3-4 fold when there is a history of PE in the family *i.e.* mother or sister (Arngrimsson *et al.,* 1990; Cincotta and Brennecke, 1998). Arngrimsson *et al.* (1990) analysed 94 families spanning over at least 3 generations, the authors reported that the prevalence of PE is higher in daughters-in-law (23% vs 10%). There are other reports which have suggested that a family history of PE in a mother, sister or both triples the risk of PE (Cincotta and Brennecke, 1998).

*Obesity*

Obesity has gradually become a huge public health issue, having profound impacts on all aspects of medicine. Unsurprisingly, it is an important risk factor for developing PE. Numerous studies have shown that an elevated BMI as a risk factor confers a 2-4 fold increase in the rate of PE (Eskenazi *et al.,* 1991; Stone *et al.,* 1994; Mittendorf *et al.,* 1996; Conde-Agudelo and Belizán, 2000; Mostello *et al.,* 2002; Duckitt and Harrington, 2005). A Norwegian population-based study, reported that a high pre-pregnancy BMI is not an independent risk factor for early PE but it increases the risk of late PE by two-fold (Odegard *et al.,* 2000).

Corroborating this evidence, another large prospective observational cohort study of more than 45,000 singleton pregnancies, which examined the association of maternal BMI with pregnancy complications, it was reported that the risk of PE was higher with increasing maternal BMI. This study found, with univariate analyses that the risk was higher for late PE compared to early disease (Syngelaki *et al.,* 2011). In another cohort study, the high BMI was stratified to assess whether this impacted the association between maternal BMI and risk of severe PE. The authors reported that there was no significant difference between four BMI categories with regard to risk of severe PE. These categories were normal (BMI 18.5-24.9) overweight (BMI 25-29.9, obese (BMI 30-39.9 and morbidly obese (BMI ≥40). The risk of severe late PE is increased once a woman enters the overweight category (Durst *et al.,* 2015).

There is evidence that maternal serum levels of adiponectin, a biomarker for obesity, in the first trimester are significantly higher in women who subsequently develop early PE than those who develop late PE and those who remain normotensive. The authors report that adiponectin levels are not related to biophysical and biochemical markers of impaired placentation such as uterine artery PI and serum PAPP-A. The altered levels of adiponectin may be secondary to an alternate pathophysiological process such as endothelial dysfunction (Nanda *et al.,* 2011).

*Pre-existing medical conditions*

There are certain medical conditions that are known to predispose a woman, when pregnant, to developing PE. These include type I diabetes mellitus, pre-existing chronic hypertension, renal disease, autoimmune diseases, such as systemic lupus erythematosis, and anti-phospholipid syndrome. Duckitt and Harrington (2005) analysed these conditions and their association with PE in a systematic review and they reported summary relative risks for PE given these pre-existing medical conditions.

***Diabetes mellitus***

Pre-pregnancy type I diabetes mellitus has been shown in studies to increase the risk of developing PE to over three times the normal population risk (Garner *et al.,* 1990; Ros *et al.,* 1998; Lee *et al.,* 2000).

***Chronic hypertension***

Pre-existing hypertension has been shown to double the risk for PE (Conde-Agudelo *et al.,* 2000). In another study, Catov *et al.* (2007), examining more than 70,000 women from the Danish National Birth Cohort, have shown that primiparous women with pre-existing hypertension are at a higher risk of developing early and more severe disease form of PE.

**Table 1.6** Relative risks of pre-existing medical conditions for the development of preeclampsia (modified from Duckitt and Harrington, 2005).

| Pre existing condition | Type of study | N | Unadjusted RR (95% CI) |
|---|---|---|---|
| **Type 1 Diabetes mellitus** | Cohort | 56,968 | 3.56 (2.54-4.99) |
| **Hypertension** | | | |
| Systolic ≥ 130mmHg at booking | Cohort | 906 | 2.37 (1.78-3.15) |
| Diastolic ≥ 80mmHg at booking | Cohort | 907 | 1.38 (1.01-1.87) |
| **Anti-phospholipid antibodies vs none** | Cohort | 1,802 | 9.72 (4.34-21.75) |
| **Anti-phospholipid antibodies** | Case control | 760 | 6.12 (0.35-108.35) |

***Autoimmune disease***

A matched case-control study showed that women who developed PE were six times more likely to have an autoimmune disease (Stamilio *et al.* 2000). The presence of anti-phospholipid antibodies (anti-cardiolipin antibodies and/or lupus anticoagulant) has been observed to significantly increase the risk of developing PE in both cohort and case-control studies (Branch *et al.,* 1989; Sletnes *et al.,* 1992; Pattison *et al.,* 1993; Yasuda *et al.,* 1995; Dreyfus *et al.,* 2001).

***Renal disease***

Davies *et al.* (1970) found that women who developed PE had a higher prevalence of renal disease was (5.3% vs 1.8%). Another study compared women with renal disease (n=69), based on a history of urinary tract infections, with a prospective control population matched for age, parity, smoking and date of delivery and has demonstrated a three-fold increase in the risk of developing PE, in women with a history of urinary tract infections compared to controls (6.7% vs 2.6%) (Martinell *et al.*,1990).

### 1.4.3 Biophysical markers

*Uterine artery Doppler*

Doppler ultrasound assessing the resistance to blood flow in the uterine arteries correlates with both histological studies and also clinical severity of PE. Initially, this screening was performed in the second-trimester using uterine artery Doppler, but it has also been shown to be effective to varying degrees all through pregnancy (O'Gorman *et al.,* 2016). This biophysical marker provides a useful non-invasive method for the assessment of the utero placental circulation. Studies have shown that a significant decrease in resistance in the spiral arteries occurs with advancing gestation, which is in keeping with physiological changes (Carbillon *et al.,* 2001). The impedance to flow continually diminishes up to 24-26 weeks of gestation due to the trophoblastic invasion of the spiral arteries and their conversion from narrow muscular vessels to low resistance wide non-muscular channels (Campbell *et al.,* 1983). This fall in impedance could also be due to hormonal effect in pregnancy on the elasticity of arterial walls.

Persistent high impedance to flow in the uterine arteries is evidence of poor placentation that manifests itself in the form of abnormal utero placental flow velocity waveforms. Histological examination of placental bed biopsies of pregnancies affected by PE are closely correlated with high resistance in uterine artery Doppler waveforms (Olofsson *et al.,* 1993). Studies have shown that impedance to flow in the uterine arteries is increased in PE as a direct result of not being able to convert maternal placental arteries into low pressure vessels (Aardema *et al.,* 2001).

***Second-trimester uterine artery Doppler***

This method of screening from PE has evolved from a blind technique, using continuous wave Doppler (Hanretty *et al.,* 1989) to real-time ultrasound in order to positively identify the appropriate vessels (Bower *et al.,* 1993a,b). The correct vessel is identified using pulsed wave Doppler and the uterine artery blood flow is distinguished from adjacent high resistance internal iliac vessels and lower resistance arcuate arteries. The use of colour flow along with pulsed wave Doppler aids the identification of the vessels of interest in order to obtain accurate measurements.

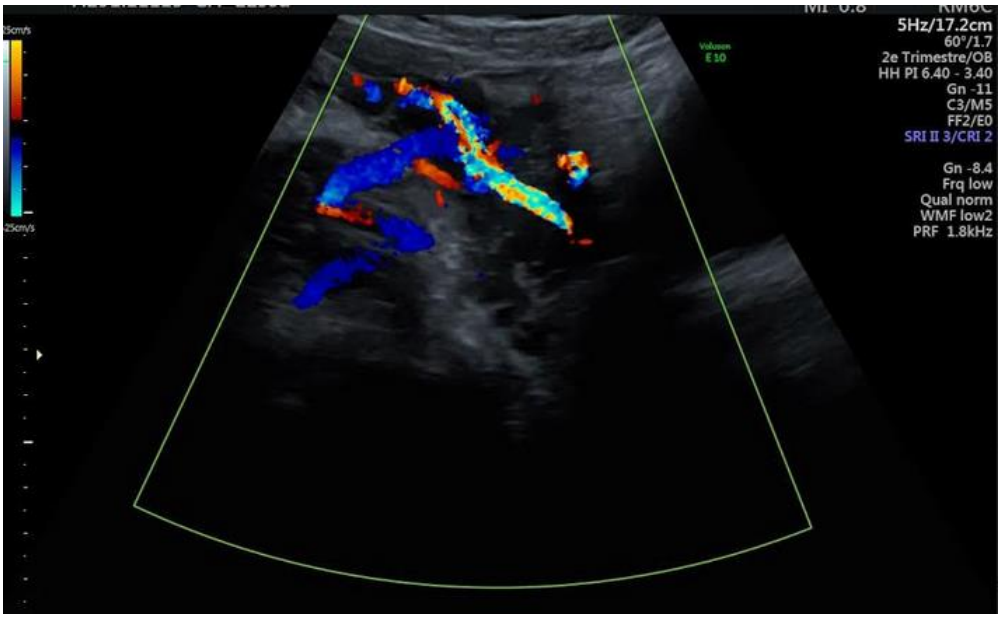


**Figure 1.4** Transabdominal technique in obtaining uterine artery waveform at the crossover with iliac artery.

The uterine artery can be identified by both transabdominal and transvaginal ultrasound. The former requires holding the transducer in the longitudinal axis and lateral to the uterus. In that position the scan shows the bifurcation of the common iliac artery into external and internal iliac arteries and there is apparent cross-over of the uterine artery and the external iliac artery (Figure 1.4) Transvaginally, the ultrasound probe is inserted into the lateral fornices and the uterine artery can be identified at the level of the internal cervical os (Figure 1.5).

The pulse wave Doppler is applied to the identified vessel and flow velocity waveform is obtained. When 3 consistent waveforms are visualised, the image is frozen and the pulsatility indices are obtained by tracing these waveforms. The indices derived from the flow velocity waveforms are described below. The resistance index ranges between 0 and 1 and that the pulsatility index is always greater than 0.

$$\textbf{Resistance index} = \frac{\textbf{(Peak systolic velocity - minimum diastolic velocity)}}{\textbf{Peak systolic velocity}}$$

**Pulsatility index = (Peak systolic velocity - minimum diastolic velocity) / Mean velocity**

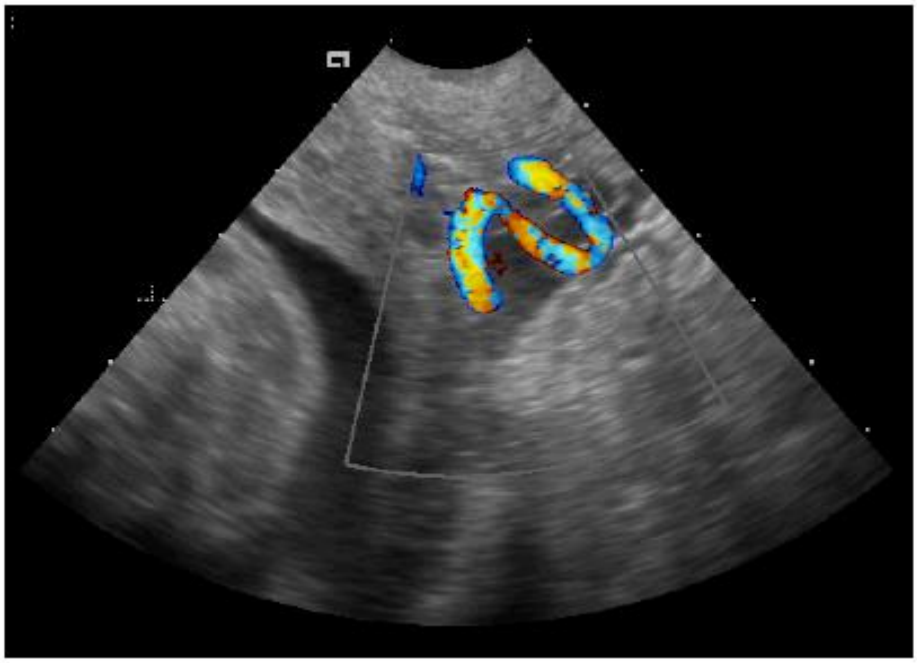

**Figure 1.5** Transvaginal technique in obtaining uterine artery waveform lateral to uterine cervix (Courtesy of Prof Nicolaides, Fetal Medicine Foundation).

When the placentation process has completed by 22-23 weeks of gestation, the normal systolic waveform of the uterine artery has a slight upslope, with an abundance of diastolic flow velocity. In contrast, the high resistance waveform is characterised by steep systolic upslope with an early diastolic notch and reduced end diastolic flow velocity (Figure 1.6). Albaiges *et al*. (2003) compared velocity and impedance indices in a high-risk population. This population was defined by an increased uterine artery impedance > 95th centile or the presence of bilateral notching at a follow-up assessment from 24 weeks of gestation. The study showed that mean impedance indices perform better than velocity indices for predicting pregnancy adverse outcomes, irrespective of whether a notch was present or not. These quantitative uterine artery indices remove the operator-dependent assessment of notches and thereby provide a more objective method of calculating the individual risk for adverse outcomes.

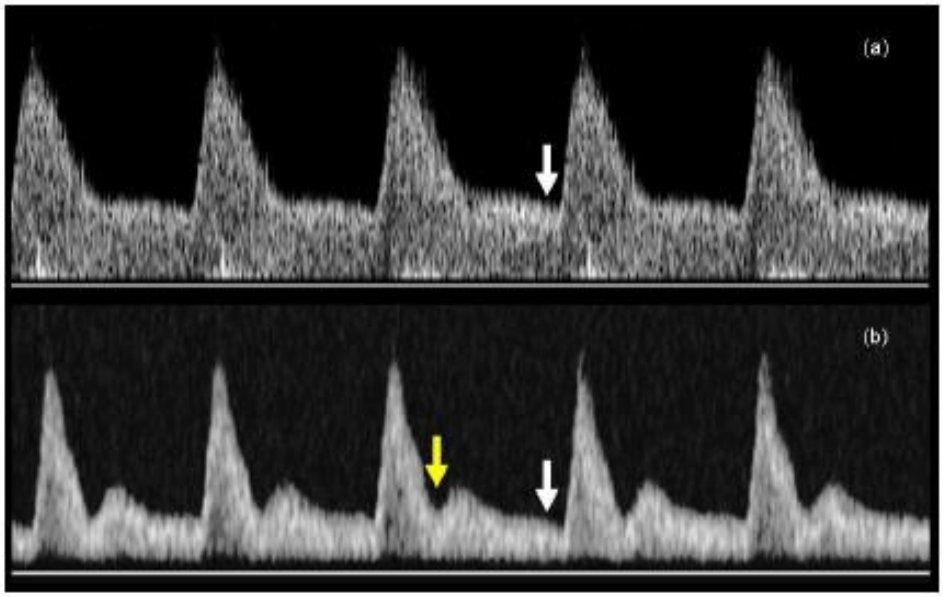


**Figure 1.6** Pulsed wave signals of uterine artery blood flow (a) normal waveform: there is a systolic upslope with an abundance of end-diastolic flow velocity (arrow); (b) abnormal waveform suggesting impaired placentation: there is a steeper systolic upslope compared with the normal waveform, with an early diastolic notch (yellow arrow) and reduced end-diastolic flow velocity (white arrow) (Courtesy of Prof Nicolaides, Fetal Medicine Foundation).

Uterine artery Doppler has been shown to be better at predicting severe disease. Bilateral uterine notching at 24 weeks of gestation can identify 55% of women who will later develop PE and 81% of those with PE requiring delivery before 35 weeks of gestation (Harrington *et al*. 1996). Kurdi *et al*. (1998) found that bilateral notches and a mean resistance index of greater than 0.55 can identify 62% of women who later developed PE and 88% with PE requiring delivery before 37weeks. Using the pulsatilty index or the presence of bilateral notching, this yielded detection rates for all PE and PE prior to 34 weeks of 45% and 90% respectively (Albaiges *et al*.,2000). In a multicentre study of 30,639 patients, the mean uterine artery PI, obtained transvaginally, was found to be above the 95$^{th}$ centile in 77.2% of women who developed PE requiring delivery before 34 weeks' gestation, in 35.9% of those delivering at 34-37 weeks' gestation and in 21.9% of those delivering after 37 weeks' gestation (Yu *et al*., 2008).

***First-trimester uterine artery Doppler***

As placentation starts in the first trimester, it appears logical that an abnormal utero-placental circulation observed in the second-trimester with uterine artery Doppler ultrasound, could also be detected with ultrasound in the first-trimester. Indeed, studies have shown that there is a good correlation between first and second-trimester uterine artery Doppler measurements.

These studies initially demonstrated that flow resistance in the uterine arteries increases with gestational age from 10 to 22 weeks and that flow in the second-trimester is influenced by the flow in the first-trimester (Jurkovic *et al.,* 1991; Kaminipetros *et al.,* 1991). The methodology and reproducibility of this apparent marker of placental dysfunction has been reported by Martin *et al.* (2001).

There are a number of factors that can influence the raw values of uterine artery Doppler pulsatility indices in pregnant women. Therefore, the uterine artery PI value needs be adjusted for these associated maternal characteristics by converting it to a multiple of the median (MoM). These associations were studied in more than 6,500 pregnancies which reported that uterine artery PI decreased with gestational age and maternal BMI and was higher in women of Afro-caribbean racial origin, in nulliparous women and, importantly, in multiparous women with a previous history of PE (Plasencia *et al.,* 2007). Taking these elements into consideration, allows a standardised approach to assessing the uterine artery PI.

A combination of maternal history and uterine artery Doppler is an effective screening method for PE, in particular early PE, with a detection rate (DR) of about 80% for a false positive rate (FPR) of 10% (Plasencia *et al.,* 2007; Poon *et al.,* 2009a). A combination of first and second trimester screening using uterine artery Doppler can improve the prediction of PE as the decrease in impedance of placental blood flow between 12 and 23 weeks is steeper in normal placentation with a normal outcome than in those that develop PE. This approach could be implemented to follow-up women with a high PI at their first trimester scan (Plasencia *et al.,* 2008).

*Blood pressure*

Although hypertension is a secondary sign of PE, it is an important sign as it is an early indication of the disease. The importance of accurate measurements of maternal blood pressure (BP) antenatally cannot be overstated. It is the mainstay of early detection, prediction and diagnosis of hypertension in pregnancy. It has already been well documented that women destined to develop PE will have elevated BP in the first- and second-trimesters of pregnancy (Moutquin *et al.,* 1985; Higgins *et al.,* 1997; Poon *et al.,* 2008 and 2011). A systematic review that assessed the value of using BP measurements to predict PE, examined data from 60,599 women including 3,341 cases with PE and concluded that mean arterial pressure (MAP) predicted PE reasonably well, with an area under the curve between 0.70 and 0.80. In contrast, systolic and diastolic BP and an increase of systolic and/or diastolic BP performed poorly in the prediction of PE with an area under the curve of <0.70 (Cnossen *et al.,* 2008). In the studies in this review, there was considerable heterogeneity which included major differences in study

design, sample size, differences in use of machines used to measure BP, mixture of high-risk and low-risk populations and major differences in the DR between studies. These are significant limitations that contributed to the finding of this review.

The use of mercury sphygmomanometers remains the gold standard for non-invasive BP monitoring. However, concerns for both their clinical performance and safety have been raised (Markandu *et al.,* 2000). Inaccurate BP readings using this method can include inter observer error and terminal digit preference, the rate at which the cuff deflates, the use the appropriate size cuff, the inter-arm difference in BP, and the arm position and posture. Automated BP monitoring will mitigate many of these obstacles and allow simple, standardised and repeated measurements to be taken. However, their use still requires both the correct cuff size and patient positioning to achieve accurate measurements.

In a large prospective screening study of more than 9,000 pregnancies at 11-13 weeks' gestation, the performance of systolic BP, diastolic BP and MAP in screening for hypertensive disorders of pregnancy using validated automated devices was assessed. Although systolic BP, diastolic BP and MAP were all found to be increased in women who developed PE, the MAP performed best as a marker with a DR for early PE increasing from 47% based on maternal factors alone to 76% based on a combination of maternal factors and MAP, at a FPR of 10% (Poon *et al.,* 2011). As for uterine artery Doppler values, the MAP should be converted into a MoM by taking into account the significant independent influencing cofactors. These include the fetal crown-rump length (CRL), maternal body mass index (BMI), maternal age, smoking status and racial origin.

Accurate measurement of MAP requires the adherence to a strict protocol (Poon *et al.,* 2012). The patient should be in the sitting position with their arms supported at the level of the heart. A small (22 cm), normal (22 to 32 cm) or large (33 to 42cm) adult cuff should be used depending on the mid-arm circumference. A validated automated device should be used, and after five minutes rest, two measurements of blood pressure should be made in both arms simultaneously and the final MAP is calculated as the average of all four measurements.

### 1.4.4 Biochemical markers

There have been numerous biochemical markers studied and evaluated for clinical use in the prediction of PE (Table 1.7). At present, there is no single marker available to accurately diagnose or predict PE, which is not surprising, given the complexity of the disorder. The alterations in the levels of these markers are the sequelae of impaired placentation secondary to inadequate trophoblastic invasion of the maternal spiral arteries and impaired placental perfusion. As mentioned above, these manifestations cause ischaemic related damage with

the release of inflammatory factors, platelet activation, endothelial dysfunction, maternal renal dysfunction or abnormal oxidative stress. Maternal serum PAPP-A and PlGF are two biochemical markers that have been investigated extensively and have shown promising results in the early prediction of PE. They have both been shown to be useful in screening for Down's syndrome at 11-13 weeks' gestation and they are now part of a platform of automated machines that provide reproducible results within 30-40 minutes of sampling.

Certain maternal and pregnancy characteristics can alter the crude serum concentration values of these metabolites and consequently, it is necessary to make adjustments for these. These include the fetal CRL, maternal weight, cigarette smoking status and ethnic origin. In addition, one must also consider the type of machine and reagents used for the assays and in order to achieve the most accurate results, the values should be expressed in multiples of the expected median (MoM) of the normal (Kagan *et al.,* 2008a).

***Pregnancy associated plasma protein-A***

PAPP-A is a metalloproteinase insulin-like growth factor (IGF) binding protein secreted by the syncytiotrophoblast that plays an important role in placental growth and development. It enhances the mitogenic function of the IGFs. PE has been shown to be associated with a low level of circulating PAPP-A, which presumably is a consequence of a reduced availability of unbound IGFs to fulfil their functional role on a cellular level. PAPP-A is a well established biochemical marker in the screening of trisomies 21, 18 and 13. It is performed in combination with maternal age, fetal nuchal translucency thickness (NT) and maternal serum free ß-human chorionic gonadotrohpin (ß-hCG) at 11-13 weeks of gestation. All three trisomies are associated with increased maternal age, increased fetal NT and decreased maternal serum PAPP-A, but in trisomy 21 serum free ß-hCG is increased whereas in trisomies 18 and 13 this is decreased. In unaffected pregnancies the median free ß-hCG and PAPP-A is 1.0 MoM. In trisomy 21 pregnancies the median free ß-hCG is 2.0 MoM and the median PAPP-A is 0.5 MoM whereas in trisomy 18 the respective values are 0.2 MoM and 0.2 MoM and in trisomy 13 they are 0.5 MoM and 0.3 MoM (Kagan *et al.,* 2008b, Nicolaides, 2011a).

In chromosomally normal pregnancies, a PAPP-A value below the 5$^{th}$ centile (0.4 MoM) is only present in 8-23% of women with PE (table 1.5). Therefore alone, this is not an accurate predictive test for PE (Yaron *et al.,* 2002; Smith *et al.,* 2002; Dugoff *et al.,* 2004; and Spencer *et al.,* 2005).

In clinical practice, when patients are found coincidentally to have a level of PAPP-A below 0.4 MoM during routine first-trimester screening for trisomies, their obstetrician should be made aware of the increased risk for PE. At this level, the reported odds ratios for PE varied between

1.5 and 4.6. Although a low PAPP-A alone is not a strong indicator of PE, but combined with second-trimester uterine artery Doppler assessment, there is a significant improvement in detection of PE (Spencer *et al.,* 2005, 2007). This had led to the recommendation that a low first trimester PAPP-A MoM in euploid pregnancies should be followed up further with 22- 24 weeks uterine artery Doppler measurement to assess the risk of PE. In established disease, there are reports suggesting that the maternal serum PAPP-A level can be increased (Bersinger *et al.,* 2003; Bersinger and Odegard, 2004; Deveci *et al.,* 2009).

**Table 1.7** Proposed maternal serum and urinary markers for the prediction of preeclampsia (modified from Conde-Agudelo *et al.*, 2004; Tjoa *et al.*, 2004; Fayyad and Harrington, 2005).

| | | | |
|---|---|---|---|
| Human chorionic gonadotropin | Placental Protein 13 | Hematocrit | Transferrin |
| Pentraxin 3 | Antiphospholipid abs | Urinary calcium excretion | Platelet activation |
| Alpha fetoprotein | Corticotropin releasing hormone | Total proteins | Haptoglobin |
| Isoprostanes | Plasminogen activator inhibitor | Urinary kallikrein | Cellular adhesion molecules |
| Estriol | Neuropeptide Y | Antithrombin III | Arial natriuretic peptide |
| Cytokines | Serum soluble fms-like tyrosine kinase | Microtransferrinuria | Endothelin |
| Inhibin A | Neurokinin B | Magnesium | β2-microglobulin |
| Homocysteine | Placental growth factor | N-acetyl-β-glucosaminidase | Prostacyclin |
| Pregnancy associated plasma protein-A | Serum uric acid | Calcium | Metabolomic markers |
| Serum lipids | Leptin | Platelet count | Thromboxane |
| Activin A | Microalbuminuria | Ferritin | Fetal DNA/RNA |
| Insulin resistance | | Fibronectin | C-reactive protein |

**Table 1.8** Summary of studies of PAPP-A in the prediction of preeclampsia.

| Author | Total | n (%) | PAPP-A cut off centile (MoM) | DR (%) | OR or RR |
|---|---|---|---|---|---|
| Ong *et al.,* 2000 | 5,297 | 135 (2.6) | 5$^{th}$ (-) | 11.1 | 2.1 |
| Yaron *et al.,* 2002 | 1,622 | 27 (1.7) | 15$^{th}$ (0.5) | 22.2 | 1.7 |
| Smith *et al.,* 2002 | 8,839 | 331 (3.7) | 5$^{th}$ (-) | 10.6 | 2.3 |
| Dugoff *et al.,* 2004 | 33,395 | 764 (2.3) | 5$^{th}$ (0.42) | 7.9 | 1.5 |
| Spencer *et al.,* 2005 | 4,390 | 64 (1.5) | 5$^{th}$ (0.42) | 14.1 | 2.8 |
| Pilalis *et al.,* 2007 | 878 | 13 (1.5) | 5$^{th}$ (0.41) | 23.1 | 1.6 |
| Spencer *et al.,* 2007 | 47,770 | 222 (0.5) | 5$^{th}$ (0.42) | 14.6 | 3.7 |
| Poon *et al.,* 2009b | 8051 | 156 (1.9) | 5$^{th}$ (0.38) | 9.6 | 2.0 |

DR = detection rate; OR = odds ratio; RR = relative risk.

***Placental growth factor***

Placental growth factor (PlGF) is a glycosylated dimeric glycoprotein secreted by trophoblastic cells and is part of the angiogenic vascular endothelial growth factor family. It binds to vascular endothelial growth factor receptor-1, which has been shown to increase in pregnancy. PlGF is synthesised in villous and extravillous cytotrophoblast and has both vasculogenetic and angiogenetic functions. Its angiogenetic abilities have been speculated to play a role in normal pregnancy and changes in the levels of PlGF or its inhibitory receptor have been implicated in the development of PE (Maynard *et al.,* 2003; Ahmad and Ahmed, 2004; Levine *et al.,* 2004; Stepan *et al.,* 2007).

**Table 1.9** Studies comparing maternal serum PlGF in pregnancies with preeclampsia and normotensive controls in the first-trimester.

| **Author** | | **Preeclampsia** | | **Controls** | | ***P*** |
|---|---|---|---|---|---|---|
| | **Gestation (w)** | **N** | **PlGF (pg/ml)** | **n** | **PlGF (pg/ml)** | |
| Tidwell *et al.,* 2001 | 5-15 | 14 | 20.1 | 25 | 58.5 | <0.0001 |
| Ong *et al.,* 2001 | 11-14 | 131 | 1.09 (MoM) | 400 | 0.98 (MoM) | NS |
| Thadhani *et al.,* 2004 | 11-14 | 40 | 23 | 80 | 63 | <0.01 |
| Erez *et al.,* 2008 | 9-14 | 17*<br>35 | 20.3<br>26.2 | 201<br>201 | 35.4<br>35.4 | 0.002<br>0.003 |
| Akolekar *et al.,* 2011 | 11-13 | 127 | 0.611* (MoM)<br>0.822 (MoM)^ | 609 | 0.991 | <0.0001 |
| Crovetto *et al.,* 2015 | 11-13 | 303 | 21.9*<br>27.5^ | 9159 | 33.4 | <0.05 |

*Early PE statistically significant, ^Late PE

Reduced levels of serum PlGF have also been shown to be present in the first-trimester of pregnancy (Table 1.9). Previous studies demonstrated that prediction of PE can be improved by combining the second-trimester uterine artery Doppler findings with maternal serum concentration of PlGF (Espinoza *et al.,* 2007) and the anti-angiogenic protein soluble fms-like tyrosine kinase 1 (sFlt-1) (Stepan *et al.,* 2007). Although reduced levels of PlGF are evident from the first-trimester, the significant increase in levels of sFlt-1 is only unmasked

approximately five weeks before the onset of PE (Levine *et al.,* 2004). The authors also found that the PlGF concentrations steadily increase with gestational age, with levels peaking at 29-32 weeks' gestation and decreasing thereafter. In women with PE, the levels follow a similar pattern but were significantly lower than in controls from as early as 12-13 weeks of gestation (Levine *et al.,* 2004).

*Screening by cell free DNA*

Following the discovery of fetal DNA in the maternal serum, several studies have reported that in women with established PE, the plasma or serum concentrations of both total and fetal cell free (cf)DNA are higher than in normotensive controls and the increase is particularly marked in those with severe PE (Lo *et al.,* 1999; Zhong *et al.,* 2001; Alberry *et al.,* 2009; Miranda *et al.,* 2013; Zeybek *et al.,* 2013). These findings have been attributed to accelerated apoptosis of trophoblastic cells resulting from placental ischaemia (Lo *et al.,* 1999) and reduced clearance of the cfDNA from the maternal circulation in women with PE (Lau *et al.,* 2002). There is conflicting data as to whether these altered levels precede the onset of the disease. A recent systematic review looked at the quantification of cfDNA for predicting PE (Martin *et al.,* 2014). The study included 3 prospective cohort studies and ten case-control studies with a total of 440 cases of PE and 2,576 controls. They found that in 11 of the 13 studies, significantly higher concentrations of fetal cfDNA were present in women who developed PE. However, in was noted that most of these studies did not adequately control for possible confounding factors such as BMI, smoking status and racial origin, and that the definitions of PE and its severity varied largely. As a result of this significant heterogeneity between the published studies, a clinically meaningful meta-analysis was not possible, and therefore no precise conclusions could be drawn. In a recent study, it was demonstrated that, at 11-13 weeks of gestation, in pregnancies that subsequently develop early-PE, the median maternal plasma concentration of total cfDNA was increased and fetal fraction was reduced. In pregnancies that developed late-PE the median fetal fraction at 20–24 weeks was reduced. However, both total cfDNA and fetal fraction were affected by maternal characteristics and when these associations were taken into account, the MoMs in the PE cases were not significantly different from normotensive controls. Thus, a beneficial consequence of applying cfDNA in the screening for PE has yet to be proved for routine clinical practice (Rolnik *et al.,* 2015).

### 1.4.5 Combined screening approach

Effective first trimester screening for PE can also be achieved using a combination of maternal factors, and the above mentioned biochemical and biophysical markers, in a similar approach to that of the combined screening test for Down's syndrome. A prospective screening study with 33,602 singleton pregnancies using a combined approach reported a prevalence of early

(<34 weeks), intermediate (34-37 weeks) and late PE (>37 weeks) to be 0.3%, 0.6% and 1.3%, respectively. This combined method of screening was performed using a logistic regression analysis to derive the *a priori* risk for each of the PE groups from maternal characteristics. The maternal factors-related *a priori* risks were then multiplied by the likelihood ratios of the biophysical and biochemical markers to derive the *a posteriori* risks and these were used to calculate the detection rates at fixed FPRs of 5 and 10%. Various algorithms which combined maternal characteristics with biophysical and biochemical tests at 11–13 weeks' gestation identified approximately 90%, 80% and 60% of pregnancies that subsequently developed early, intermediate and late PE, at a FPR of 5% (Akolekar et al., 2011).

### 1.4.6 Quality control of biomarkers

The development of quality assurance and quality control systems in prenatal care has arisen as a result of the strive for excellence and self-improvement, the need to deliver the best possible care, and the increasing impact of legal aspects in obstetrics and ultrasound practice.

Early advances in quality control originated from the nuchal translucency (NT) screening programme (Wojdemann *et al*., 2001; Snijders *et al*., 2002). This was a consequence of a large variation in screening efficiency between centres, illustrating that conditions under which the measurement is performed should be clearly defined. This approach led to both quantitative and qualitative forms of control. The quantitative approach compares the distribution of measurements taken with reference values (Snijders *et al*., 1998; Snijders *et al*., 2002). A qualitative control mechanism assesses an individual's performance, through a process of audit. Such an audit involves both assessment of the distribution of measurements for a given sonographer as well as examination of the quality of random images from each sonographer. If the criteria are met, the sonographer may be given a certification of competence.

There is particularly relevant in the context of screening for PE as each of the biochemical and biophysical markers are susceptible to inaccurate measurements, thus impacting on the risk given to the patient. The quality of the biomarkers can be assessed by close surveillance of their median MoMs using a cumulative sum (CUSUM) chart (Figure 1.7). The CUSUM chart allows detection of both increases and decreases in a process within parameter of interest. Deviations can be flagged up and addressed in a timely manner in order to avoid profound adverse inaccuracies in the screening system. Inaccurate biochemical marker results may occur as a result of a change in batch of reagent used, a change in temperature, deviation from the manufacturer's protocol, and failure to implement a continuous quality control process.

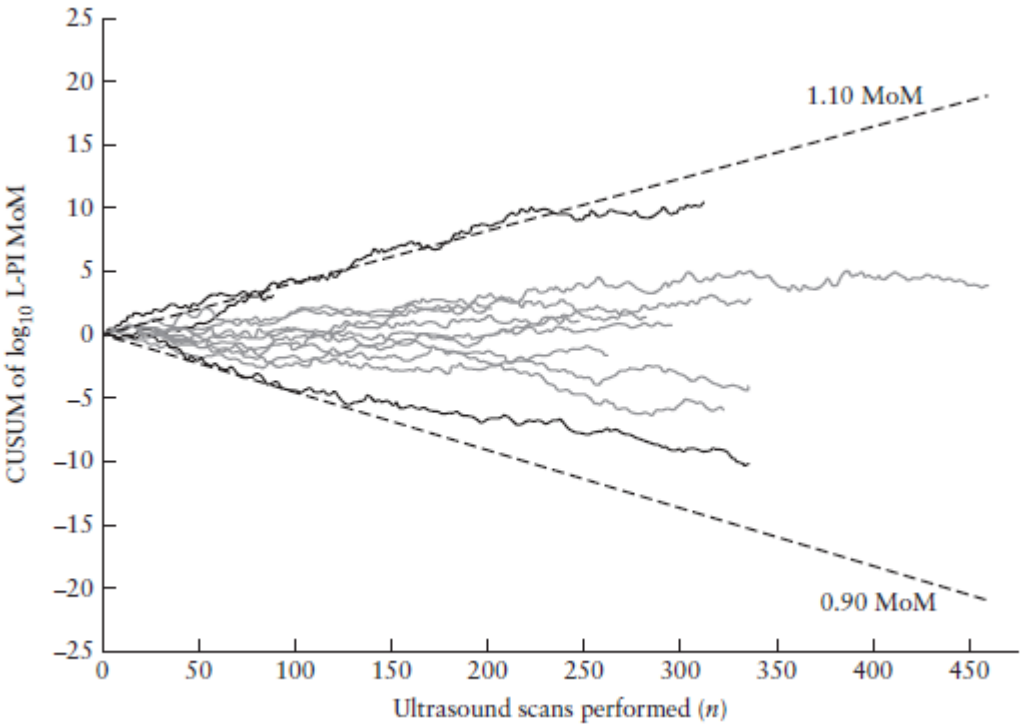


**Figure 1.7** Example of a CUSUM plot.

This chart plots the lowest uterine artery pulsatility index (L-PI) measurements of each sonographer over the course of a study. Sonographers whose measurements were not consistently within the 0.90–1.10-MoM control range are indicated by black lines (Taken from Ridding *et al.,* 2015a).

The biophysical markers MAP and uterine artery PI are equally susceptible to extensive variability in measurement. This results from poor adherence to a well-defined protocol. The quality control of the uterine artery Doppler PI has been shown to be more accurate in a group of sonographers who received feedback on their performance than those without any feedback (Ridding *et al.,* 2015a). In addition, the researchers found that the screen positive rate was also significantly higher (4.3% vs 6.8%; p=0.012) in the no-feedback group.

Other studies have demonstrated that measurements distal to the correct sampling site (Khalil and Nicolaides, 2013c) result in lower PI values (Lefevbre *et al.,* 2012; Ridding *et al.*, 2015b). Operators with a low central tendency (median MoM<0.95) may be sampling the uterine artery too distally in a significant proportion of patients. In contrast, for operators who tend to overestimate the MoM (>1.05), the measurement acquisition is unknown and although never formally tested, it is suspected that the uterine artery PI value is from a sampling site proximal to the recommended site.

## 1.5 Factors that may reduce the risk of preeclampsia

Although there is no proven effective treatment or prevention for this multisystem disorder, the physician must make a decision in the best interests of both the mother and the fetus. The target is to prolong the pregnancy for as long as is safely possible, to minimise the adverse effects of fetal prematurity. In doing so, there is a risk of worsening of the maternal disease, which in turn could potentially compromise both mother and baby. There are several possible factors that have been investigated and used in order to try to prevent PE. These include various lifestyle measures, dietary supplementation and pharmacological treatment.

### 1.5.1 Lifestyle measures

Regular exercise in pregnancy, in addition to reducing long-term obesity, is perceived to have beneficial effects in reducing the risk of developing PE. This physical activity can also reduce markers of insulin resistance and endothelial dysfunction that are known to be elevated in PE. However, exercise could in theory, be associated with an increased risk of PE as it may be associated with increased oxidative stress (Osterdal *et al.,* 2009), one of the above mentioned potential pathophysiological mechanisms of the disorder. Consequently, conflicting opinions are divided between health-care professionals on exercise during pregnancy.

A study reported in 2003 retrospectively examined 201 women with PE and 383 women with normotensive patients (Sorensen *et al.,* 2003). Women were given a structured questionnaire regarding the sort, duration, frequency and intensity of exercise both during pregnancy, and in the year preceding the pregnancy. The study reported an overall 35% reduced risk (95% CI 0.43 to 0.99) of PE, corrected for age, BMI, parity, smoking and race, in women who exercised regularly in the first 20 weeks of pregnancy, compared to inactive women. The risk of PE was inversely related to the frequency and intensity of exercise. Interestingly, women who had undertaken regular exercise in the year preceding pregnancy had a similar risk reduction to those who exercised during pregnancy (Odds ratio (OR) 0.67, 95% CI 0.42 - 1.08).

Another study evaluated the impact of exercise on the risk of preeclampsia. The authors distributed a questionnaire to pregnant women between the gestational weeks 14 and 22, and reported an overall reduction in risk of preeclampsia with exercise of 21% (95% CI 0.65 - 0.96). However, this effect was most marked in women with a normal BMI, and no beneficial effect of exercise was seen with pregnancy in those with a BMI >30 kg/$m^2$. This led the authors to believe that the beneficial effects of exercise in pregnancy only applied to the non-obese population (Magnus *et al.,* 2008).

A review of the literature in this field led the American College of Obstetrics and Gynaecology (ACOG Committee Opinion, 2002) to recommend that in the absence of medical or obstetric complications, pregnant women should perform 30 minutes of moderate exercise daily.

Similarly, the NICE guidance was that starting or continuing a moderate course of exercise is not associated with adverse outcomes (Kenyon *et al.,* 2007). A recent randomised control study with 765 patients divided women into two groups to assess whether exercise protects against hypertension and macrosomia. The group randomised to exercise trained 3 days per week for 50 to 55 minutes per session from 9-11 until 38-39 weeks' gestation. The 85 training sessions involved aerobic exercise, muscular strength, and flexibility. The authors reported that high attendance to this programme, regardless of BMI, that pregnant women who did not exercise are 3 times more likely to develop hypertension (OR 2.96; 95% CI, 1.29-6.81, P=0.01) and are 1.5 times more likely to gain excessive weight (OR 1.47; 95% CI 1.06-2.03, P=0.02). In addition, the lack of exercise increases the risk 2.5 times to give birth to a macrosomic infant (OR 2.53; 95% CI 1.03-6.20, P=0.04) (Barakat *et al*., 2016).

### 1.5.2 Calcium supplementation

It has been suggested that calcium supplementation reduces high BP. Low calcium intake may elevate BP by stimulating either parathyroid hormone or renin release, which results in increased intracellular calcium in vascular smooth muscle causing vasoconstriction (Belizan *et al.*, 1988). In 1952 it was reported that Ethiopian women, who had a diet rich in calcium, had a low prevalence of PE (Hamlin, 1952). In the calcium to prevent preeclampsia (CPEP) double blinded randomised control trial, 4589 healthy nulliparous women, at 13 to 21 weeks' gestation, were randomised to either 2g of calcium daily or a placebo. There was no significant difference in rates of PE (6.9% vs 7.3% (RR 0.94; 95% CI 0.76-1.16)), gestational hypertension (77% vs 76%, RR 1.04; 95% CI 0.74-1.38), preterm deliveries (10.8% vs 10%), or in perinatal mortality (34% vs 36%) in the calcium and placebo group respectively.

A Cochrane database systematic review involving 14 studies that included 15,730 women reported that calcium supplementation of more than 1g per day was associated with a reduction in risk of PE (Relative risk (RR) 0.45, 95% CI 0.31-0.65), particularly for women with low-calcium diets (RR 0.36, 95% CI 0.20-0.65). The validity of these promising results, however, had limitations as the results could have been potentially confounded by the inclusion of several small trials with a high proportion of women with low dietary calcium intake. This is evident from the significant amount of heterogeneity between these studies (Hofmeyr *et al.,* 2014). It therefore remains uncertain whether calcium supplementation helps reduce the risk of PE.

### 1.5.3 Anti-oxidants

As oxidative stress has been associated with the pathophysiology of PE, there have been studies which have examined whether anti-oxidants administration would reduce the production of reactive oxygen species (ROS) and in turn, oxidative stress (Vaughan and

Walsh, 2002). This could in theory impact the development of PE. Anti-oxidants play a key role in maintaining cellular integrity in normal pregnancy. They do this by inhibiting peroxidation reactions which protects vital enzyme action, proteins and cells from destruction by peroxides (Rumbold *et al.,* 2006). The studies that have investigated the role of anti-oxidants in the prevention of PE have mostly involved the use of vitamins C and E. The former, vitamin C (ascorbic acid) scavenges free radicals in the aqueous phase, and the lipid soluble vitamin E ($\alpha$-tocopherol) acts *in vivo* to prevent the formation of lipid peroxides and thereby, protects cell membranes (Rumbold *et al.*, 2006). Diet supplementation of these vitamins may reduce the oxidative stress by preventing endothelial damage and the subsequent development of PE.

A meta-analysis of seven studies that included 5,969 women, concluded that concomitant supplementation with vitamins C and E does not prevent PE in women at risk (pooled RR: 0.79; 95% CI 0.58-1.08), but actually increases the rate of babies born with a low birth weight (pooled RR: 1.13; 95% CI of 1.00-1.27) (Rahmi *et al.,* 2009).

Magnesium supplementation in pregnancy has also been implicated as a possibility of reducing the incidence of PE (Conradt *et al.,* 1984). Unfortunately, a Cochrane review of two randomised trials (474 patients) showed no apparent effect of magnesium treatment on the risk of PE or GH (Makrides and Crowther, 2001).

Another anti-oxidant, which has been investigated in regard to prevention of PE is Zinc. It is an essential trace element important for many metabolic pathways. A reduction in maternal and umbilical plasma zinc concentrations has been seen in women with PE when compared to normal pregnancy (Bassiouni *et al.,* 1979). This is possibly due to the fact that zinc and oestrogen compete for common binding sites on plasma proteins (Adeniyi, 1987). These findings led to a recommended maintenance of adequate dietary zinc nutrition during pregnancy. Two randomised controlled trials involving 1,288 patients, however, failed to demonstrate any significant benefit (Mahomed *et al.,* 1989; Jonsson *et al.,* 1996).

### 1.5.4 Folate

There are both animal and human studies which promote the role of folic acid in the prevention of PE (Wen *et al.,* 2008a and 2008b). The mechanisms to explain this effect include a contribution to placental growth and development, by lowering blood homocysteine levels and, through the effect of folic acid in improving endothelial function.

Hyperhomocysteinemia is a risk factor for PE and by reducing plasma levels of homocysteine with folic acid, it is hypothesised that this may reduce the risk of PE (Lindblad *et al.,*2005; Guven *et al.,* 2009). Increased levels of homocysteine also can lead to endothelial activation and folic acid may mitigate this endothelial dysfunction thereby contributing to the prevention of PE (Powers *et al.,* 1998).

There is data from four retrospective cohort studies which examined the effect of folic acid in the prevention of PE which suggest that regular use of folic acid supplementation reduces the risk of PE (Hernandez-Diaz *et al.,* 2002; Bodnar *et al.,* 2006; Wen *et al.,* 2008a; Catov *et al.,* 2009). There are two other subsequent studies which failed to illustrate a protective effect of folic acid supplementation (Timmermans *et al.,* 2011; Li *et al.,* 2013). A systematic review brought these studies together to find an OR of 0.14 (95% CI 0.06-0.31) for a reduction in risk of PE, showing a strong protective effect of folic acid supplementation (Wen *et al.,* 2013).

There is currently an international multicentre RCT in progress to examine the effectiveness of folic acid in the prevention of PE. This study, 'The Effect of Folic Acid Supplementation in Pregnancy on Preeclampsia: the Folic Acid Clinical Trial (FACT) aims to recruit 3,656 high risk women to evaluate whether supplementation of folic acid throughout pregnancy will impact the development of PE. Those patients categorised into the high-risk group will be randomised in a 1:1 ratio to either folic acid 4.0 mg or placebo between 8 and 16 weeks of gestation will (Wen *et al.,* 2013).

### 1.5.5 Heparin

As discussed above, the placenta in pregnancies affected with PE are associated with thrombosis, necrosis and infarction. Consequently, it has been postulated that heparin, which is a large complex macromolecule, could be used to mitigate these pathological changes and potentially prevent development of the disorder. There has been much interest in investigating the use of low molecular weight heparin (LMWH) alone or in combination with an anti-platelet drug for prevention of miscarriage, PE, and intrauterine growth restriction (IUGR) in pregnancy, especially in women with a known thrombophilic tendency or a previous history of PE. However, there are studies that have been too small and underpowered for reliable conclusions (Duley *et al.,* 2006a; 2006b; Walker *et al.,* 2006).

Conversely, two systematic reviews summarising the published literature concluded that heparin does significantly reduce the recurrence of PE and is associated with reductions in perinatal mortality, preterm delivery, and growth restriction (<$10^{th}$ percentile) in high-risk women (Dodd *et al.,* 2013; Rodger *et al.,* 2014). Roberge *et al.* (2015) reported in their meta-analysis that there is some evidence that using LMWH with aspirin in women with a history of PE compared to aspirin alone was associated with a significant reduction in PE (RR 0.54, 95% CI 0.31-0.92) (Roberge *et al.,* 2015). However, LMWH used alone vs no treatment was evaluated in a study of 135 participants and did not a significant reduction in adverse perinatal outcome (risk difference, 2.2 (95% CI 11.6-16.0); P = 0.76 (Martinelli *et al.,* 2012). Similarly, the TIPPS trial including 292 women showed that LMWH without aspirin had no effect on adverse outcome (Rodger *et al.,* 2014).

The exact mechanism of action of LMWH and how it potentially prevents PE remains elusive but it is likely to be attributable to the anti-coagulant action of heparin within the placenta. Another hypothesis is that LMWH could exert a direct vascular action in the maternal compartment to reverse the placenta-mediated systemic vascular dysfunction characteristic of PE (McLaughlin *et al.,* 2015). It has also been shown that LMWH can significantly reduce systolic and diastolic blood pressure and can reduce vascular resistance in the utero placental circulation (Mello *et al.,* 2005). Although, there is some evidence for a its potential role in preventing PE in high-risk women, large high quality RCTs are necessary to corroborate the results of smaller studies and to define the extent of any possible benefit in reducing the prevalence of PE.

### 1.5.6 Aspirin

Research into the use of low-dose aspirin (acetylsalicylic acid) for the prevention of PE has been ongoing for the last 30 years. Crandon and Isherwood (1979) observed that nulliparous women who had taken aspirin regularly during pregnancy were less likely to have PE than those who did not. Aspirin exerts its effect by targeting prostaglandin pathways and modifying the imbalance between thromboxane A2 and prostacyclin, which is altered in PE and contributes to the vasospasm and coagulation abnormalities. In normal pregnancy, prostacyclin concentrations increase and cause a reduction in systemic vascular resistance by reducing the maternal response to angiotensin II and other vasopressors (Wallenburg *et al.,* 1991). In PE, the ratio of thromboxane A2 to prostacyclin is disrupted as, eicosanoid synthesis is greatly altered with relatively low prostacyclin levels. This imbalance leads to the augmentation of the pressor effects of angiotensin II and catecholamines, resulting in the clinical syndrome of PE (Chavarria *et al.,* 2003).

Low-dose aspirin treatment in pregnancy is thought to prevent the development of PE by inhibiting the biosynthesis of placental thromboxane A2 with minimal effects on vascular prostacyclin levels (Sibai *et al.,* 2005). The enzyme cyclo-oxygenase (COX) plays a pivotal role in the production of both prostacyclin and thromboxane A2. Aspirin inhibits endothelial cyclo-oxygenase (Dekker and Sibai, 2001) and this process is irreversible in platelets, where the enzyme is inhibited for their entire life-span. In contrast, when the enzyme is re-synthesised in endothelial cells, the prostacyclin production is re-established relatively rapidly. This selective inhibition of cyclo-oxygenase and the resulting alteration in the prostacyclin to thromboxane A2 ratio in the placenta, forms the basis of using aspirin to prevent or delay the onset of PE.

In the wake of Crandon and Isherwood's study, more reports emerged in the literature suggesting that low dose aspirin in high-risk women reduces the prevalence of fetal growth

restriction and PE (Beaufils *et al.,* 1985; Wallenburg *et al.,* 1986). Following this, there were a number of large randomised studies that showed aspirin had no effect on PE (Italian Study of Aspirin in Pregnancy, 1993; CLASP Collaborative Group, 1994). It is believed that the negative outcome of these studies was due to the low risk population selection.

A subsequent meta-analysis, which examined more than 30,000 women from 39 trials, reported that the use of an anti-platelet agent was associated with a 15% reduction in the risk of PE (32 trials; 29,331 women; pooled RR 0.85) with an 8% reduction in the risk of preterm birth before 37 weeks and a 14% reduction in the risk of fetal or neonatal death in women allocated to low dose aspirin (Duley *et al.,* 2001). The PARIS (Perinatal Antiplatelet Review of International Studies) collaborative group (2005) examined individual patient data from 32,217 women from 31 randomised trials of antiplatelets for the prevention of PE. They found that women assigned to receive anti-platelet treatment, had a relative risk (RR) of developing PE, of delivering before 34 weeks and of having a pregnancy with a serious adverse outcome of 0.90. Their findings were similar to those of Duley *et al.* (2004) in that there was a moderate but consistent reduction in the relative risk of PE (Askie *et al.,* 2007).

A number of randomised studies have examined the value of prophylactic aspirin in women with increased impedance to flow in the uterine arteries. A meta-analysis examining nine of these studies, with a total of 1,317 women, assessed the influence of gestational age at the time of administration of low dose aspirin on the incidence of PE. These women were categorised as high risk, based on an abnormal uterine artery Doppler ultrasound. This study showed that aspirin treatment commenced in early gestation was, in fact, associated with a greater reduction in the incidence of PE than treatment started in late gestation: <16 weeks of gestation resulted in a RR of 0.48 (95% CI 0.33-0.68), at 17-19 weeks RR 0.55 (95% CI 0.17-1.76), and at > 20 weeks RR 0.82 (95% CI 0.62-1.09) (Bujold *et al.,* 2009). The same group performed another meta-analysis with 11,348 patients and found again in 9 RCTs with 764 patients, that aspirin prescribed prior to 16 weeks' gestation was significantly associated with a reduction in PE (RR 0.47, 95% CI 0.34-0.65) and IUGR (RR0.44, 95% CI 0.3-0.65). Aspirin started after 16 weeks' gestation in 18 RCTs with 10,584 women, was not associated with a reduction in the incidence of PE or IUGR (RR 0.81 95% CI 0.63-1.03 and RR 0.98, 95% CI 0.87-1.1 respectively) (Bujold *et al.,* 2010).

The authors subsequently examined the effect of early administration of aspirin from 5 studies with 556 patients and reported that it was particularly effective in preventing early PE requiring delivery before 37 weeks' gestation, rather than at term (RR 0.11, 95% CI 0.04-0.33 vs RR 0.98, 95% CI 0.42-2.33) (Roberge *et al.,* 2012a). In another meta-analysis, 4 studies with 392 women, severe PE, as opposed to mild PE was significantly decreased with aspirin given prior

to 16 weeks' gestation (RR 0.22, 95% CI 0.08-0.57 vs RR 0.81, 95% CI 0.33-1.96, respectively) (Roberge *et al.,* 2012b). Additionally, they highlighted there was also a 50% reduction in the risk of IUGR and 60% reduction in the risk of perinatal death if treatment was commenced before 16 weeks rather than there after (Roberge *et al.,* 2013; Bujold *et al.,* 2014).

More recently, a retrospective analysis of two cohorts who screened high risk for PE in the first trimester, showed a significant reduction in the incidence of both early and late PE in the cohort of women prescribed 150mg of aspirin following screening ($p<0.01$ and $p=0.03$, respectively) (Park *et al*., 2015). It has been widely postulated that aspirin's mechanism of action particularly benefits those who are at high risk of early PE as a consequence of improved placentation. However, a triple blinded randomised control trial which gave either 150mg of aspirin or a placebo to patients with abnormal uterine artery Dopplers in the first trimester, found no difference in the mean uterine artery PI at 28 weeks' gestation. The only true way to answer the question as to whether aspirin prevents PE is to conduct an adequately powered double blinded randomised control trial which following an effective first trimester screening programme, either aspirin or placebo is prescribed and the pregnancy outcome recorded.

# CHAPTER 2 PATIENTS AND METHODS

## 2.1 Study population

The first study, the development of the competing risks model, presented in chapter 3 is based on data collected from two UK hospitals as a part of the prospective screening study for hypertensive disorders. The second and third studies in chapter 3, the validation study and the comparison study, are based on data collected from 12 maternity hospitals in five different EU countries, as part of a prospective first trimester screening quality study for preeclampsia.

### 2.1.1 The competing risks model

This study was a part of an ongoing prospective screening study for early prediction of pregnancy complications in women attending their first routine hospital visit in pregnancy at King's College Hospital, London, and Medway Maritime Hospital, Gillingham, between February 2010 and July 2014.

At this appointment, which is held between $11^{+0}$ and $13^{+6}$ weeks' gestation, all women have an ultrasound scan to confirm the gestational age using the measurement of the fetal crown rump length (CRL), to diagnose the presence of any major fetal abnormalities and to measure fetal nuchal translucency (NT) thickness and maternal serum free β-human chorionic gonadotrophin (b-hCG) and PAPP-A as part of screening for chromosomal abnormalities (Snijders *et al*., 1998; Kagan *et al*., 2008b).

All women attending for this visit were invited to participate in the research study entitled 'Early Prediction of Pregnancy Complications'. Written informed consent was obtained from those agreeing to participate in the study. We subsequently recorded maternal characteristics and medical and obstetric history, measured the uterine artery pulsatility index (PI) by transabdominal colour Doppler, mean arterial pressure (MAP) (section 2.4) using a validated automated device and stored plasma and serum at -80$^{o}$C for subsequent biochemical analysis.

The inclusion criteria for this study were singleton pregnancies delivering a phenotypically normal live birth or stillbirth at or after 24 weeks' gestation. We excluded pregnancies with major fetal abnormalities and those ending in termination, miscarriage or fetal death before 24 weeks' gestation.

**Table 2.1.** Maternal and pregnancy characteristics in the total screening population of the competing risks model.

| Variables | Preeclampsia (n=1,058) | Unaffected (n=34,890) | *p* value |
|---|---|---|---|
| Maternal age in years, median (IQR) | 31.5 (27.0, 35.6) | 31.3 (26.8, 35.0) | 0.34501 |
| Maternal weight in kg, median (IQR) | 72.1 63.0, 86.7) | 66.5 (59.0, 77.0) | 0.37555 |
| Maternal height in cm, median (IQR) | 163.2 (159, 168) | 164.5 (160, 169) | 0.19445 |
| Body mass index, median (IQR) | 27.1 (23.5, 32.1) | 24.5 (21.9, 28.3) | 0.66575 |
| Gestational age in weeks, median (IQR) | 12·6 (12·3, 13·0) | 12·7 (12·3, 13·1) | 0.19424 |
| Racial origin | | | <0.0001 |
| Caucasian, n (%) | 564 53.3%) | 25,315(72.6%) | |
| Afro-Caribbean, n (%) | 394 (37.2%) | 6,287 (18%) | |
| South Asian, n (%) | 56 (5.3%) | 1,567 (4.5%) | |
| East Asian, n (%) | 17 (1.6%) | 829 (2.4%) | |
| Mixed, n (%) | 27 (2.6%) | 892 (2.6%) | |
| Medical history | | | |
| Chronic hypertension | 140 (13.2%) | 421 (1.2%) | <0.0001 |
| Diabetes mellitus | 22 (2.1%) | 303 (0.9%) | 0·0008 |
| SLE/APS | 5 (0.5%) | 48 (0.1%) | 0.01679 |
| Cigarette smokers, n (%) | 68 (6.4%) | 3,195 (9.2%) | 0.00278 |
| Family history of preeclampsia, (n, %) | | | |
| Parity | | | <0.0001 |
| Nulliparous, n (%) | 622 (58.8%) | 16,739 (48%) | |
| Parous with no previous PE, n (%) | 283 (26.8%) | 17,028 (48.8%) | |
| Parous with previous PE, n (%) | 153 (14.5%) | 1,123 (3.2%) | |

IQR = interquartile range; SLE = systemic lupus erythematosus; APS = antiphospholopid syndrome
Comparisons between outcome groups were by chi-square or Fisher exact test for categorical variables and Mann Whitney-U test for continuous variables; * significance value p<0.05

In this screening study, the competing risks model, described in Chapter 3, we screened 35,948 women with 1,058 (2.9%) cases that developed PE and 34,890 that were unaffected by PE. All women had a complete history taken with the biomarkers MAP, uterine artery PI, PAPP-A, and PlGF. In the PE group, compared to unaffected pregnancies, there was no significant difference in maternal age, weight, and BMI. There was, however, a higher mean prevalence of Afro-Caribbean racial origin, family and previous history of PE, chronic hypertension, diabetes mellitus and systemic lupus erythematosus or antiphospholipid syndrome and, as previously published, a lower prevalence of cigarette smokers.

### 2.1.2 The validation study

This was a prospective, non-interventional, multicentre validation study of the competing risks model (described above in section 2.1.1) in screening for preeclampsia by maternal factors and biomarkers at 11-13 weeks' gestation. The study was comprised of singleton pregnancies at $11^{+0}$-$13^{+6}$ weeks' gestation in women booking for routine pregnancy care at the following hospitals; King's College Hospital, London, UK, Medway Maritime Hospital, Gillingham, UK, Homerton University Hospital, London, UK, North Middlesex University Hospital, London, UK, Southend University Hospital, Essex, UK, Lewisham University Hospital, London, UK, Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain, Hospital Universitario San Cecilio, Granada, Spain, Hospiten Sur, Tenerife, Spain, Centre Hospitalier Universitaire Brugmann, Brussels Belgium, Attikon University Hospital, Athens, Greece and Ospedale Maggiore Policlinico, Milan, Italy.

All women undergoing routine screening for aneuploidies in these hospitals were invited to participate in the screening study for preeclampsia. In women who agreed to participate in the study, the maternal MAP was measured by automated devices (Poon *et al.,* 2012), and transabdominal colour Doppler ultrasound was used to visualise the left and right uterine artery, to measure the PI in each vessel and calculate the mean PI (Plasencia *et al.,* 2007). Maternal serum samples were taken via standard venepuncture for the analysis of PAPP-A and PlGF using a Delfia Xpress automated machine.

The patients who attended for their first trimester ultrasound between 11-13 weeks' gestation were recruited between February and September 2015 and gave written informed consent to participate in the study, which was approved by the NHS Research Ethics Committee in the UK and the Ethics Committee of each participating hospital in other countries.

The inclusion criteria for this study required patients to be aged 18 years or more, singleton pregnancy, and have a live fetus at the time of screening. Those with multiple pregnancies, major abnormalities identified at the ultrasound scan, those deemed to lack capacity to be able to consent to participate, and pregnancies ending in termination or miscarriage, were excluded from the study.

During the study period, 9,041 pregnancies meeting the inclusion criteria underwent screening for PE. The maternal demographics can be seen in table 2.2. We subsequently excluded 266 (2.9%) cases because they had a major fetal defect (n=33), the pregnancy resulted in termination (n=39) or miscarriage (n=88) or there was no follow up (n=106).

In the PE affected group, compared to unaffected pregnancies, there was no significant difference in maternal age, smoking status and systemic lupus erythematosus or antiphospholipid syndrome. As in the competing risks model, we did observe a higher prevalence of Afro-Caribbean racial origin, those with a family and personal history of PE, chronic hypertension, diabetes mellitus and a lower prevalence of cigarette smokers.

### 2.1.3 The comparison study

This study used the data obtained from the above prospective validation study to directly compare the detection rates of preeclampsia using our competing risks model with the screening strategies recommended by the National Institute of Health and Clinical Excellence (NICE, 2010) and the American College of Obstetricians and Gynecologists (ACOG, 2015).

## 2.2 Recording of information

Prior to seeing a doctor, women were asked to complete a questionnaire on age, racial origin (Caucasian, African, South Asian, East Asian and Mixed), cigarette smoking during pregnancy (yes or no), method of conception (spontaneous or assisted conception by either ovulation induction alone or in vitro fertilisation), medical history (including chronic hypertension, diabetes mellitus, anti-phospholipid syndrome, thrombophilia and sickle cell disease), medication (including anti-hypertensive, anti-depressant, anti-epileptic, aspirin, steroids, betamimetic, insulin, and thyroxine), parity (parous, nulliparous with no previous pregnancies, nulliparous with miscarriage or termination before 24 weeks), previous pregnancy with PE (yes or no) and family history of PE (mother, sister or both). The questionnaire was then reviewed by a doctor and the medical and obstetric history was recorded on an electronic database.

The following measurements were taken:

- Maternal weight and height were measured and the BMI was calculated in $Kg/m^2$,
- Stabilised measurement of systolic BP, diastolic BP and MAP,
- Uterine artery PI of the two arteries,
- Measurement of maternal serum PAPP-A and PlGF concentrations.

### 2.3 Ethical committee approval

The screening study which resulted in the competing risks model was a sub study of the Early Prediction of Pregnancy Complications Study, which was approved by the King's College Hospital Ethics Committee (Ref: 02-03-033, R&D reference number 03WH06). The patients received an information leaflet outlining the details of the research study (appendix 1) and written informed consent (appendix 2) was obtained from the women agreeing to participate in the study.

The prospective validation and comparison study was part of an FP7 EU funded study entitled Combined Multi-Marker Screening and Randomised Patient Treatment with Aspirin for Evidence-based Pre-eclampsia (ASPRE). A favourable opinion was granted by the National Research Ethics Service (NRES) Committee London – Fulham (Ref 13/LO/1479). As with the screening study above, the potential participants received a patient information sheet (appendix 3) providing an overview of the study and those that agreed to partake in the research, signed a consent form (appendix 4) agreeing to the terms and conditions of the study.

## 2.4 Biochemical markers

Patient blood samples were collected for all patients who provided written consent agreeing to take part in the research. The blood samples were collected in two 10ml tubes [red (plain) and purple (EDTA)]. The tubes were labelled with a patient identifying barcode and transferred to the laboratory. The samples were processed in the laboratory after a standing time of 10-15 minutes at room temperature to allow for clotting. The samples were centrifuged at 3000rpm for 10 minutes to separate serum and plasma. 0.5ml of the serum was separated into a falcon and placed in the DELFIA® Xpress (DX) analyser for the biochemistry results.

### 2.4.1 Pregnancy associated plasma protein-A

Maternal serum pregnancy associated plasma protein-A (PAPP-A) was measured using a DELFIA® XPRESS analyzer (PerkinElmer Life and Analytical Sciences, Waltham, USA). This is solid-phase, two-site fluoroimmunometric assay that is based on the indirect sandwich technique. The assay utilises monoclonal antibodies immobilised on microplates which are directed against the PAPP-A protein. During the elution process, PAPP-A binds to the coated wells and interacts with europium tagged monoclonal antibodies. The addition of an enhancement solution causes dissociation of the tag from the antibody where they form highly fluorescent chelates with components of the enhancement solution. The fluorescence is proportional to the concentration of PAPP-A in the sample and its concentration can be measured. The measured maternal serum PAPP-A was converted to multiples of the expected normal median (MoM) corrected for fetal CRL, maternal age, weight, smoking, parity, racial origin and method of conception as previously described (Kagan *et al.*,2008a).

### 2.4.2 Placental growth factor

The assay used for the competing risk model was the DELFIA® Xpress PlGF assay. This is a solid phase, two-site fluoroimmunometric assay in which monoclonal antibodies (derived from mice) and polyclonal antibodies (derived from rabbits) are directed against the PlGF molecule

and is based on the direct sandwich technique. Patient blood samples, containing PlGF, are reacted with immobilised polyclonal antibodies directed against the PlGF. Europium-labelled monoclonal antibodies directed against an antigenic site on the PlGF are reacted with the PlGF bound to the solid-phase antibody. DELFIA® Inducer dissociates europium ions from the labelled antibodies in the sample, where they form highly fluorescent chelates with components of the DELFIA® Inducer. The fluorescence in each cup is then measured. The europium fluorescence from each sample is proportional to the concentration of PlGF in the sample.

The DELFIA® Xpress PlGF 1-2-3 assay was used for the validation and comparison studies. This is a newer assay with the main difference being that it uses two monoclonal antibodies. The second monoclonal antibody attaches to the PlGF with the europium tag and its fluorescence can be measured to produce a PlGF concentration. The measured maternal serum PlGF was converted to multiples of the expected normal median (MoM) corrected for fetal CRL, weight, smoking, and racial origin (Akolekar *et al.*, 2008).

**Table 2.2** Maternal and pregnancy characteristics in the validation and comparison study

| Maternal characteristics | Delivery with preeclampsia | | | |
|---|---|---|---|---|
| | None (n=8,536) | <32 (n=17) | <37 (n=59) | ≥37 (n=180) |
| Maternal age in years, median (IQR) | 31.5 (27.3, 35.0) | 29.8 (26.7, 34.6) | 30.6 (25.95, 34.7) | 31.2 (27.8, 34.8) |
| Maternal weight in Kg, median (IQR) | 66.2 (58.8, 76.9) | 72.6 (65.6, 86.0) | 69.8 (63.0, 87.8) | 75.0 (64.9, 84.0) |
| Maternal height in cm, median (IQR) | 165 (160, 169) | 164 (161, 166) | 164 (160, 169) | 164 (159, 168) |
| Body mass index, median (IQR) | 24.5 (21.9, 28.3) | 27.3 (23.9, 31.8) | 27.1 (23.6, 31.82) | 27.8 (23.9, 31.5) |
| Gestational age in weeks, median (IQR) | 12.7 (12.3, 13.1) | 12.6 (12.3, 12.7) | 12.7 (12.4, 13.0) | 12.7 (12.3, 13.2) |
| Racial origin, n (%) | | | | |
| Caucasian | 6,716 (78.7) | 8 (47.1) | 38 (64.4) | 129 (71.7) |
| AfroCaribbean | 1,040 (12.2) | 8 (47.1) | 14 (23.7) | 36 (20.0) |
| East Asian | 153 (1.8) | 0 (0.0) | 0 (0.0) | 1 (0.6) |
| South Asian | 447 (5.2) | 0 (0.0) | 3 (5.1) | 12 (6.7) |
| Mixed | 180 (2.1) | 1 (5.9) | 4 (6.8) | 2 (1.1) |
| Medical history, n (%) | | | | |
| Chronic hypertension | 75 (0.9) | 3 (17.7) | 9 (15.3) | 16 (8.9) |
| Diabetes mellitus | 63 (0.7) | 2 (11.8) | 3 (5.1) | 2 (1.1) |
| SLE or APS | 32 (0.4) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Cigarette smoking, n (%) | 717 (8.4) | 1 (5.9) | 4 (6.8) | 11 (6.1) |
| Family history of preeclampsia, n (%) | 434 (5.1) | 1 (5.9) | 7 (11.9) | 17 (9.4) |
| Conception, n (%) | | | | |
| Spontaneous | 8,254 (96.7) | 17 (100) | 57 (96.6) | 173 (96.1) |
| *In vitro* fertilization | 218 (2.6) | 0 (0.0) | 2 (3.4) | 7 (3.9) |
| Ovulation drugs | 64 (0.7) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Parity, n (%) | | | | |
| Nulliparous | 3,972 (46.5) | 11 (64.7) | 36 (61.0) | 119 (66.1) |
| Parous: no previous preeclampsia | 4,396 (51.5) | 4 (23.5) | 17 (28.8) | 46 (25.6) |
| Parous: previous preeclampsia | 168 (2.0) | 2 (11.8) | 6 (10.2) | 15 (8.3) |
| Pregnancy interval in years, median (IQR) | 2.7 (1.6, 4.6) | 5.4 (4.3, 7.2) | 4.1 (2.4, 6.8) | 3.4 (2.0, 5.4) |

IQR = interquartile range; SLE = systemic lupus erythematosus; APS = antiphospholipid syndrome; Comparisons between outcome groups were by chi-square or Fisher exact test for categorical variables and Mann Whitney-U test for continuous variables

## 2.5 Biophysical markers

### 2.5.1 Blood pressure

The blood pressure (BP) was taken by validated automated devices (Microlife Watch BP Home, Taipei, Taiwan; Chung, 2009) that were calibrated before and at regular intervals during the study (every 1,000 inflations). The recordings were made by doctors or midwives or researchers who had received appropriate training on the use of these machines. Women were allowed to rest for 5-10 minutes and the BP measurements were taken in a quiet room with

temperature of between 20°C and 24°C. The women were seated, with their arms supported at the level of the heart and either a small (< 22cm), normal (22-32 cm) or large (33-42 cm) adult cuff was used depending on their mid arm circumference (Pickering *et al.,* 2005; Poon *et al*., 2012). The BP was measured in both arms simultaneously (Figure 2.1) and 2 recordings of BP were taken and the MAP was calculated using an average of the two systolic and diastolic readings. A patient with BP above 140/90mmHg was referred to the antenatal clinic for obstetric management as per the local protocol.

**Figure 2.1** Simultaneous measurement of blood pressure in both arms

### 2.5.2 Uterine artery Doppler

The measurement of uterine artery PI by transabdominal ultrasound required obtaining a sagittal section of the uterus and the visualisation of both the cervical canal and the internal cervical os. The transducer was then gently tilted from side to side and colour flow mapping was used to identify each uterine artery along the side of the cervix and uterus at the level of the internal os (Figure 2.2; Plasencia *et al.,* 2007). Pulsed wave Doppler was used with the sampling gate set at 2 mm to cover the whole vessel and care was taken to ensure that the angle of insonation was less than 30º. When three similar consecutive waveforms were obtained, the uterine artery PI was measured from the left and right arteries (Figure 2.3). All sonographers obtained the Fetal Medicine Foundation Certificate of competence in obstetric Doppler imaging prior to their involvement in the study (http://www.fetalmedicine.com).The results of the Doppler studies were not given to the women or their doctors and did not influence the subsequent management of the pregnancies.

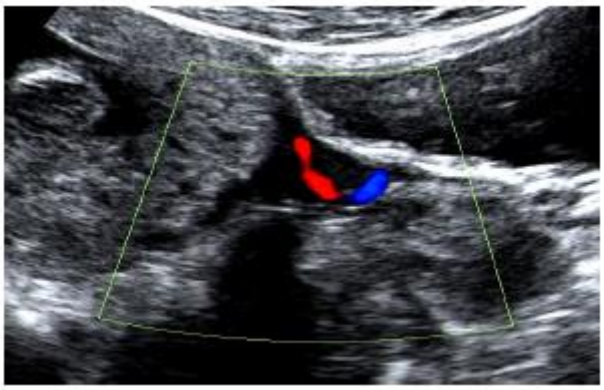

**Figure 2.2** The uterine artery as demonstrated by colour flow mapping along the side of the cervix and uterus at the level of the internal os.

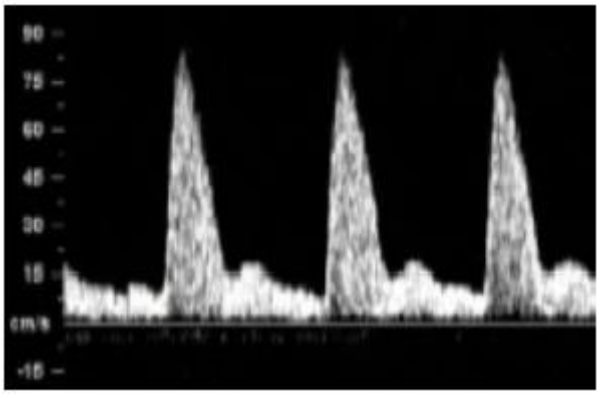


**Figure 2.3** First trimester uterine artery waveform

## 2.6 Outcome measures

The definitions used for PE and GH were those of the International Society for the Study of Hypertension in Pregnancy (Davey and MacGillivray, 1988). In GH the diastolic BP should be 90 mmHg or more on at least two occasions four hours apart from 20 weeks' gestation in previously normotensive women in the absence of significant proteinuria. A diagnosis of PE was made if there was GH with proteinuria of 300 mg or more in a given 24 hour period or two readings of at least ++ on dipstick analysis of midstream or catheter urine specimens (if no 24-hour collection was available). In PE superimposed on chronic hypertension significant proteinuria (as defined above) should develop after 20 weeks' gestation in women with known chronic hypertension (*ie* a history of hypertension before conception or the presence of hypertension at the booking visit before 20 weeks' gestation in the absence of trophoblastic disease).

The obstetric medical records of all women with pre-existing or pregnancy associated hypertension were examined to differentiate definitively whether the condition was chronic hypertension, PE or GH.

## 2.7 Statistical analysis

### 2.7.1 The competing risks model

This first trimester screening model for PE is based on a survival time model for the time of delivery for PE. Bayes theorem was used to combine maternal demographic and past medical history in formation with biochemical and biophysical markers that were converted into multiple of the median (MoM) values. We used the competing risks model described by (Kalbfleisch and Prentice, 2002). In this model, it is assumed that if the pregnancy was to continue indefinitely all women would develop PE. Therefore, there is a competition between the delivery with PE and delivery for other reasons (Figure 2.4). The model was applied to represent the distribution of gestational age at delivery with PE. In pregnancies at low risk of PE, the gestational age distribution is shifted to the right which implies that actually most pregnancies will deliver before the onset of PE. Conversely, in those at high risk of developing PE, the gestational age distribution at which they will develop PE is shifted to the left yielding a greater area under the curve before 42 weeks' gestation with the implication that an increased number of women will deliver will actually occur after the development of PE.

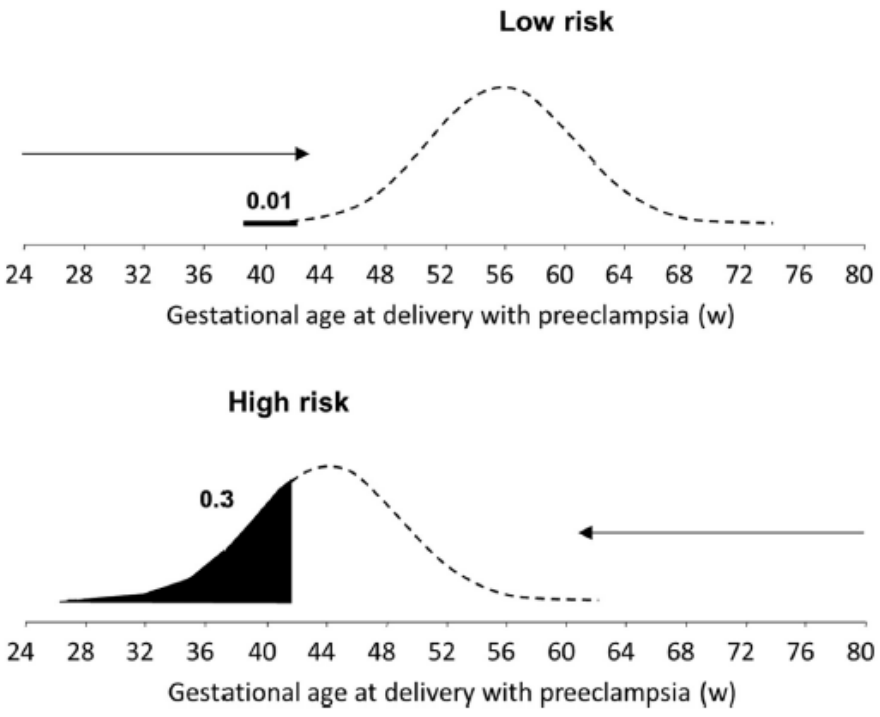


**Figure 2.4** The Competing risks model (taken from Wright *et al.,* 2015).

The distribution of gestational age at delivery with PE was obtained by applying Bayes theorem to combine the prior distribution based on maternal characteristics with likelihoods of the time to delivery with PE given the biophysical and biochemical marker MoM values as above. Although such model fitting was complicated because of the censoring in situations when delivery occurred before PE, fitting regression models to censored data was carried out routinely with standard statistical software. For example, a delivery without PE at 37 weeks' gestation would be classed as a censored observation, meaning that the PE event occurred after 37 weeks' gestation. A delivery with PE at 37 weeks' gestation is an observation at 37 weeks' gestation that is not censored. In the analysis, the outcome data for each individual comprises of a gestation at delivery and a variable indicating whether delivery was with or without PE.

The values of uterine artery PI, MAP, PAPP-A and PLGF were $\log_{10}$ transformed to achieve homogeneity of variants and approximate Gaussian distributions around the mean. Each measured value in the unaffected and PE pregnancies was expressed as a MoM after adjusting for confounding characteristics (Wright *et al.,* 2010 and 2012; Pandya *et al.,* 2012). In each case of PE, the measurements were converted into a MoM and regression analysis was used to determine the relationship between $\log_{10}$ MoM values with gestational age at delivery.

In the estimation of performance of screening the values of uterine artery PI, MAP, PAPP-A and PLGF in the whole screened population were simulated based on the mean and SD of the $\log_{10}$ transformed marker values in the unaffected and PE pregnancies. In the PE group the mean and SD values used for simulation were specific for each gestational week at delivery, which were estimated from the regression analysis of the $\log_{10}$ MoM values of available data with gestational age at delivery.

### 2.7.2 The validation and comparison studies

The previously described competing risks model algorithm above was used for calculation of patient-specific risk of delivery with PE <32, <37 and ≥37 weeks' gestation in the 8,775 patients in the validation study. The pre-specified analyses for performance of screening by maternal factors and any combinations of maternal factors with MAP, uterine artery PI, PAPP-A and PlGF were estimation of areas under the receiver–operating characteristics curve (AUROC) and detection rates (DR), with 95% CI, at false positive rates (FPR) of 5% and 10%. The essential difference between the above competing risk model and the validation study is that the testing of the competing risks model was performed on the data set used to develop the

model. To test the model a five-fold cross validation was used in which the data set was randomly divided into one fifths and the data in each fifth was fitted into the model of the other 4/5. This approach was taken to limit the degree of optimistic bias. The validation study used a new data set to prospectively validate the competing risk model. McNemar's test was used for the comparison study to compare the screen positive rates in our data set between the competing risks model and the screening approach recommended by NICE and ACOG. The statistical software package R was used for data analyses (R Development Core Team, 2011).

# CHAPTER 3 PUBLICATIONS

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This chapter incorporates the following publications in peer reviewed journals:

- O'Gorman N, Wright D, Syngelaki A, Akolekar R, Wright A, Poon LC, Nicolaides KH. 2016. Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 11-13 weeks gestation. *Am J Obstet Gynecol* 214: 103 e101-103 e112. DOI 10.1016/j.ajog.2015.08.034.

- O'Gorman N, Wright D, Poon LC, Rolnik DL, Syngelaki A, Wright A, Akolekar R, Cicero S, Janga D, Jani J, Molina FS, de Paco Matallana C, Papantoniou N, Persico, N, Plasencia W, Singh M. Nicolaides KH. 2017. Accuracy of competing-risks model in screening for pre-eclampsia by maternal factors and biomarkers at 11–13 weeks' gestation. Ultrasound Obstet Gynecol, 49: 751–755. doi:10.1002/uog.17399.

- O'Gorman N, Wright D, Poon LC, Rolnik DL, Syngelaki A, de Alvarado M, Carbone, IF, Dutemeyer V, Fiolna M, Frick A, Karagiotis N, Mastrodima S, de Paco Matallana C, Papaioannou G, Pazos A, Plasencia W, Nicolaides KH. 2017. Multicenter screening for pre-eclampsia by maternal factors and biomarkers at 11–13 weeks' gestation: comparison with NICE guidelines and ACOG recommendations. *Ultrasound Obstet Gynecol* 49: 756–760. doi:10.1002/uog.17455.

OBSTETRICS

# Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 11-13 weeks gestation

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**BACKGROUND:** Preeclampsia affects approximately 3% of all pregnancies and is a major cause of maternal and perinatal morbidity and death. In the last decade, extensive research has been devoted to early screening for preeclampsia with the aim of reducing the prevalence of the disease through pharmacologic intervention in the high-risk group starting from the first trimester of pregnancy.

**OBJECTIVE:** The purpose of this study was to develop a model for preeclampsia based on maternal demographic characteristics and medical history (maternal factors) and biomarkers.

**STUDY DESIGN:** The data for this study were derived from prospective screening for adverse obstetric outcomes in women who attended for their routine first hospital visit at 11-13 weeks gestation in 2 maternity hospitals in England. We screened 35,948 singleton pregnancies that included 1058 pregnancies (2.9%) that experienced preeclampsia. Bayes theorem was used to combine the a priori risk from maternal factors with various combinations of uterine artery pulsatility index, mean arterial pressure, serum pregnancy-associated plasma protein-A, and placental growth factor multiple of the median values. Five-fold cross validation was used to assess the performance of screening for preeclampsia that delivered at <37 weeks gestation (preterm-preeclampsia) and ≥37 weeks gestation (term-preeclampsia) by models that combined maternal factors with individual biomarkers and their combination with screening by maternal factors alone.

**RESULTS:** In pregnancies that experienced preeclampsia, the values of uterine artery pulsatility index and mean arterial pressure were increased, and the values of serum pregnancy-associated plasma protein-A and placental growth factor were decreased. For all biomarkers, the deviation from normal was greater for early than late preeclampsia; therefore, the performance of screening was related inversely to the gestational age at which delivery became necessary for maternal and/or fetal indications. Combined screening by maternal factors, uterine artery pulsatility index, mean arterial pressure, and placental growth factor predicted 75% (95% confidence interval, 70-80%) of preterm-preeclampsia and 47% (95% confidence interval, 44-51%) of term-preeclampsia, at a false-positive rate of 10%; inclusion of pregnancy-associated plasma protein-A did not improve the performance of screening. Such detection rates are superior to the respective values of 49% (95% confidence interval, 43-55%) and 38% (34-41%) that were achieved by screening with maternal factors alone.

**CONCLUSION:** Combination of maternal factors and biomarkers provides effective first-trimester screening for preterm-preeclampsia.

**Key words:** Bayes theorem, first trimester screening, mean arterial pressure, placental growth factor, preeclampsia, pregnancy-associated plasma protein-A, uterine artery

Preeclampsia affects 2-3% of all pregnancies and is a major cause of maternal and perinatal morbidity and death.[1,2] In the last decade extensive research has been devoted to screening for preeclampsia with the aims of (1) to reduce the prevalence of the disease through pharmacologic intervention in the high-risk group[3,4] and (2) to minimize adverse perinatal events for those who experience preeclampsia by the determination of the appropriate time and place for delivery.[5] The traditional approach to screening for preeclampsia

EDITORS' CHOICE

is to identify risk factors from maternal demographic characteristics and medical history (maternal factors), but such an approach can identify only 35% of all preeclampsia and approximately 40% of preterm-preeclampsia, at false-positive rate (FPR) of 10%.[6,7]

An alternative approach to screening, which allows estimation of individual patient- specific risks of preeclampsia that requires delivery before a specified gestation, is to use Bayes theorem to combine the a priori risk from maternal characteristics and medical history (maternal factors) with the results of various combinations of biophysical and biochemical measurements that are made at different times during pregnancy.[8,9] We adopted this approach using a competing risk model for the time to delivery with preeclampsia. This model assumes that, if the pregnancy was to continue indefinitely, all women would experience preeclampsia; whether they do so before a specified gestational age depends on competition between delivery before or after the development of preeclampsia.[8] The effect of maternal factors is to modify the mean of the distribution of gestational age at delivery with preeclampsia so that, in pregnancies that are at low-risk for preeclampsia, the gestational age distribution is shifted to the right with the implication that, in most pregnancies, delivery actually will occur before the development of preeclampsia. In high-risk pregnancies the distribution is shifted to the left, and the smaller the mean gestational age, the higher is the risk for preeclampsia. The distribution of biomarkers is specified conditionally on the gestational age at delivery with preeclampsia. For any women with specific maternal factors

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and biomarker multiple of the median (MoM) values, the posterior distribution of the time to delivery with preeclampsia, assuming that there is no other cause of delivery, is obtained from the application of Bayes theorem.

We have reported previously on the development and performance of a maternal factor–derived algorithm for the prediction of preeclampsia.[7] We have also proposed a model for combining the maternal factor–derived previous risk with the results of uterine artery pulsatility index (PI), mean arterial pressure (MAP), serum placental growth factor (PLGF), and pregnancy-associated plasma protein-A (PAPP-A).[8,9] However, the performance of screening was assessed by simulation from the fitted model, and such an approach generally is biased optimistically because it ignores errors of estimation and departures from the assumed model.

The objective of this study of 35,948 singleton pregnancies, which included 1058 patients (2.9%) who experienced preeclampsia, with complete data on uterine artery PI, MAP, serum PLGF, and PAPP-A, is to examine the potential improvement in performance of screening by maternal factors alone[7] with the addition of each biomarker and combinations of biomarkers. Performance of screening was assessed with the use of 5-fold cross validation.

## Methods

### Study population

The data for this study were derived from prospective screening for adverse obstetric outcomes in women who were attending for their routine first hospital visit in pregnancy at King's College Hospital and Medway Maritime Hospital, UK. This visit, which was held at $11^{+0}$ to $13^{+6}$ weeks gestation, included (1) the recording of maternal characteristics and medical history,[7] (2) measurement of the left and right uterine artery PI by transabdominal color Doppler ultrasound scanning and calculation of the mean PI,[10] (3) measurement of MAP by validated automated devices and standardized protocol,[11] and (4) measurement of serum concentration of PLGF and PAPP-A (DELFIA Xpress system, PerkinElmer Life and Analytical Sciences, Waltham, MA). Gestational age was determined from the fetal crown-rump

**TABLE 1**
**Maternal and pregnancy characteristics in the screening population**

| Variables | Unaffected (n = 34,890) | Preeclampsia (n = 1058) | *P* value |
|---|---|---|---|
| Maternal age, y[a] | 31.3 (26.8–35.0) | 31.5 (27.0–35.6) | .34501 |
| Maternal weight, kg[a] | 66.5 (59.0–77.0) | 72.1 (63.0–86.7) | .37555 |
| Maternal height, cm[a] | 164.5 (160.0–169.0) | 163.2 (159.0–168.0) | .19445 |
| Body mass index, kg/m$^2$[a] | 24.5 (21.9–28.3) | 27.1 (23.5–32.1) | .66575 |
| Gestational age, wk[a] | 12.7 (12.3–13.1) | 12.7 (12.3–13.1) | .19424 |
| Racial origin, n (%) | | | < .00001 |
| White | 25,315 (72.6) | 564 (53.3) | |
| Afro-Caribbean | 6,287 (18.0) | 394 (37.2) | |
| South Asian | 1,567 (4.5) | 56 (5.3) | |
| East Asian | 829 (2.4) | 17 (1.6) | |
| Mixed | 892 (2.6) | 27 (2.6) | |
| Medical history | | | |
| Chronic hypertension | 421 (1.2) | 140 (13.2) | < .00001 |
| Diabetes mellitus | 303 (0.9) | 22 (2.1) | .00008 |
| Systemic lupus erythematosus/antiphospholipid syndrome | 48 (0.1) | 5 (0.5) | .01679 |
| Cigarette smokers, n (%) | 3,195 (9.2) | 68 (6.4) | .00278 |
| Family history of preeclampsia, n (%) | 1,428 (4.1) | 90 (8.5) | < .00001 |
| Parity, n (%) | | | < .00001 |
| Nulliparous | 16,739 (48.0) | 622 (58.8) | |
| Parous with no previous preeclampsia | 17,028 (48.8) | 283 (26.8) | |
| Parous with previous preeclampsia | 1,123 (3.2) | 153 (14.5) | |

Comparisons between outcome groups were by chi-square or Fisher exact test for categoric variables and Mann-Whitney *U* test for continuous variables.

[a] Data are given as median (interquartile range).

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**TABLE 2**
**Fitted regression model for marker $log_{10}$ multiple of the median values on gestation at time of delivery for pregnancies with preeclampsia**

| Marker | Intercept | Standard error | Slope | Standard error | *P* value |
|---|---|---|---|---|---|
| Uterine artery pulsatility index | 0.54453 | 0.05300 | −0.013143 | 0.001401 | < .0001 |
| Mean arterial pressure | 0.095640 | 0.014420 | −0.0018240 | 0.0003811 | < .0001 |
| Pregnancy associated plasma protein-A | −0.62165 | 0.09721 | 0.014692 | 0.002569 | < .0001 |
| Placental growth factor | −0.93687 | 0.07573 | 0.021930 | 0.002002 | < .0001 |

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length.[12] The women were screened between February 2010 and July 2014 and gave written informed consent to participate in the study, which was approved by the NHS Research Ethics Committee.

The inclusion criteria were singleton pregnancy undergoing first-trimester combined screening for aneuploidy and subsequently delivering a phenotypically normal live birth or stillbirth at ≥24 weeks gestation. We excluded pregnancies with aneuploidies and major fetal abnormalities and those ending in termination, miscarriage, or fetal death before 24 weeks gestation.

## Outcome measures

Data on pregnancy outcome were collected from the hospital maternity records or the general medical practitioners of the women. The obstetric records of all women with preexisting or pregnancy-associated hypertension were examined to determine whether the condition was preeclampsia, as defined by the International Society for the Study of Hypertension in Pregnancy.[13]

## Statistical analyses

Our model for the gestational age at delivery with preeclampsia was defined by 2 components: first, the previous

**TABLE 3**
**Standard deviations and correlations for $log_{10}$ multiples of the median biomarker values**

| Variable | No preeclampsia (95% confidence interval) | Preeclampsia (95% confidence interval) | Pooled (95% confidence interval)[a] |
|---|---|---|---|
| Standard deviation | | | |
| Uterine artery pulsatility index | 0.12852 (0.12757−0.12948) | 0.14234 (0.13653−0.14868) | 0.12894 (0.12801−0.12989) |
| Mean arterial pressure | 0.03719 (0.03691−0.03746) | 0.03873 (0.03715−0.04045) | 0.03724 (0.03697−0.03751) |
| Pregnancy-associated plasma protein-A | 0.23457 (0.23284−0.23632) | 0.26108 (0.25042−0.2727) | 0.23539 (0.23368−0.23712) |
| Placental growth factor | 0.17645 (0.17515−0.17777) | 0.20141 (0.19318−0.21038) | 0.17723 (0.17595−0.17854) |
| Correlation | | | |
| Uterine artery pulsatility index, mean arterial pressure | −0.05132 (−0.06178− −0.04085) | −0.05229 (−0.11223−0.00803) | −0.05133 (−0.06163− −0.04101) |
| Uterine artery pulsatility index, pregnancy-associated plasma protein-A | −0.16039 (−0.1706− −0.15015) | −0.14735 (−0.20582− −0.08784) | −0.15992 (−0.16998− −0.14983) |
| Uterine artery pulsatility index, placental growth factor | −0.14953 (−0.15977− −0.13925) | −0.18512 (−0.24271− −0.12623) | −0.15084 (−0.16093− −0.14072) |
| Mean arterial pressure, pregnancy-associated plasma protein-A | −0.00565 (−0.01614−0.00484) | 0.01349 (−0.04685−0.07373) | −0.00497 (−0.01531−0.00537) |
| Mean arterial pressure, placental growth factor | −0.02969 (−0.04017− −0.0192) | 0.02101 (−0.03933−0.08121) | −0.02791 (−0.03824− −0.01758) |
| Pregnancy-associated plasma protein-A, placental growth factor | 0.31983 (0.31037−0.32923) | 0.34729 (0.2931−0.39925) | 0.32085 (0.31154−0.3301) |

[a] Estimates obtained from pooling data for the preeclampsia and no-preeclampsia groups.
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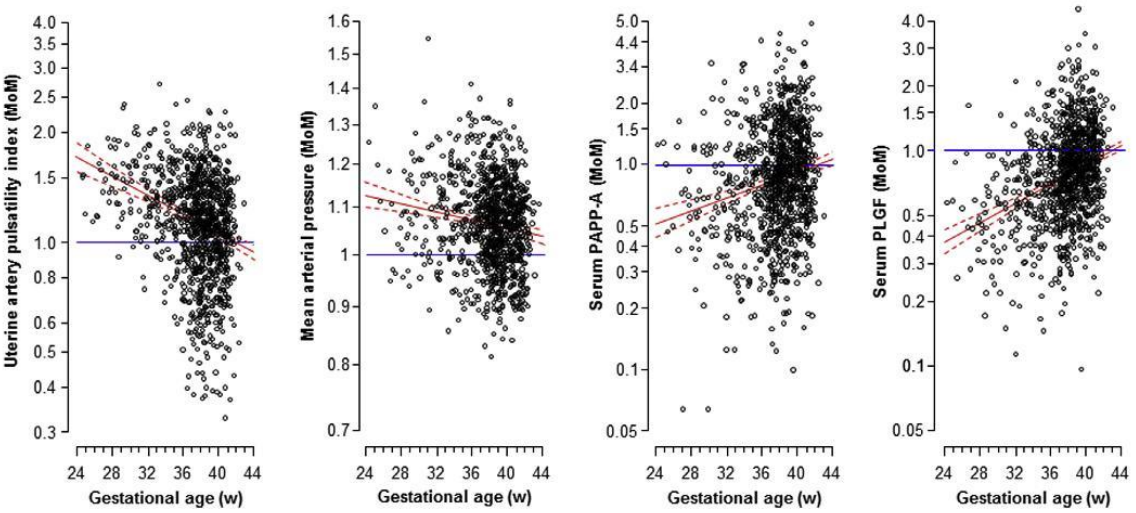
**FIGURE 1**
**Scatter diagram and regression line**




Data show the 95% confidence limits for the relationship between uterine artery pulsatility index, mean arterial pressure, serum pregnancy-associated plasma protein-A and placental growth factor multiple of the median and gestational age at delivery in pregnancies with preeclampsia.

*MoM*, multiple of the median; *PAPP-A*, pregnancy-associated plasma protein-A; *PLGF*, placental growth factor; *w*, week.

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distribution based on maternal factors[9] and, second, the conditional distribution of MoM biomarker values, given the gestational age with preeclampsia and maternal factors. Values of uterine artery PI, MAP, PAPP-A, and PLGF were expressed as a MoM adjustment for those characteristics that were found to provide a substantive contribution to the $\log_{10}$ transformed value that included the maternal factors in the previous model.[14-17] In the preeclampsia group, the mean $\log_{10}$ MoM was assumed to depend linearly with gestational age at delivery; this linear relationship was assumed to continue until the mean $\log_{10}$ MoM of zero, beyond which the mean was taken as zero. Multivariable Gaussian distributions were fitted to the $\log_{10}$ MoM values of the biomarkers, and a common covariance matrix was assumed for these distributions. Analysis of residuals was used to check the adequacy of the model and assess the effects of maternal factors on $\log_{10}$ -transformed MoM values in pregnancies with preeclampsia.

Five-fold cross validation[18] was used to assess the performance of screening for preeclampsia that delivered at <37 weeks gestation (preterm-preeclampsia), ≥37 weeks (term-preeclampsia), and subgroups of preeclampsia that delivered at <32, $32^{+0}$-$36^{+6}$, $37^{+0}$-$39^{+6}$, and ≥40 weeks by models that combined maternal factors with individual biomarkers and their combination with screening by maternal factors alone.[9] The data were divided into 5 equal subgroups; the model was then fitted 5 times to different combinations of 4 of the 5 subgroups and used to predict a risk of preeclampsia in the remaining one-fifth of the data. In each case, the maternal factor model, the regression models, and the covariance matrix were fitted to the training data set comprising four-fifths on the data and used to produce risks for the hold out sample that comprised the remaining one-fifth of the data.

The following screening strategies were considered: (1) the mini-combined test that comprised maternal factors, MAP and PAPP-A; (2) the biophysical test that comprised maternal factors, uterine artery PI, and MAP; (3) the biochemical test that comprised maternal factors and serum PLGF and PAPP-A, and (4) the quadruple test that comprised maternal factors and all 4 biomarkers. This choice covers the different combinations likely to be considered in clinical practice: the mini-combined test includes the least expensive biochemical and biophysical measurements; the biophysical and biochemical test may be preferred in ultrasound scanning only or laboratory-only settings, respectively; and the quadruple test combines all 4 markers. For each combination of biomarkers, backward elimination was used to determine the subset of biomarkers that contributed to the screening performance.

The statistical software package R was used for data analyses.[19] The survival package[20] was used for fitting the maternal factors model, and the package pROC[21] was used for the receiver operating characteristic curve analysis.

## Results

The characteristics of the study population are summarized in Table 1.

### Distribution of preeclampsia according to gestational age at delivery

In the study population, there were 1058 pregnancies that experienced preeclampsia. The gestational age at delivery of these pregnancies was <32 weeks in 66 cases (6.2%), $32^{+0}$-$36^{+6}$ weeks in 226 cases (21.4%), $37^{+0}$-$39^{+6}$ weeks in 514 cases (48.6%) and ≥40 weeks in 252 cases (23.8%). Therefore, 292 of the cases (27.6%) of preeclampsia delivered at <37 weeks, and 766 cases (72.4%) delivered at ≥37 weeks.

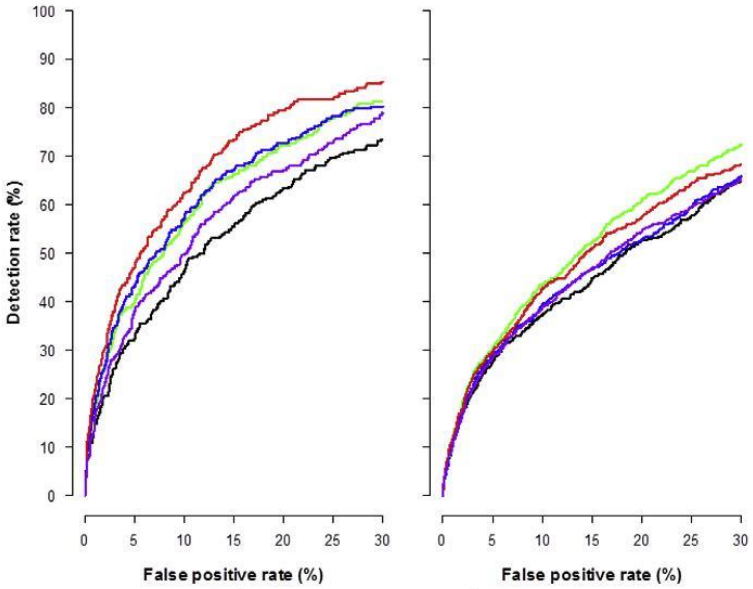
### Distribution of preeclampsia in parous and nulliparous women

In 17,361 of the 35,948 pregnancies (48.3%), the women were nulliparous; in 18,587 pregnancies (51.7%), they were parous, which included 1276 women (6.9%) with a history of preeclampsia in a previous pregnancy and 17,311 women (93.1%) without a history of preeclampsia. In the current pregnancy, preeclampsia occurred in 1058 cases (2.9%), which included 292 cases (0.8%) of preterm-preeclampsia and 766 cases (2.1%) of term-preeclampsia. The contribution of parous women was 45.2% (132/292) to preterm-preeclampsia and 39.7% (304/766) to term-preeclampsia; the respective values were 35.6% (47/132) and 34.9% (106/304) for parous women with preeclampsia in a previous pregnancy and 64.4% (85/132) and 65.1% (198/304) for parous women without a history of preeclampsia.

### Distribution of biomarkers

The distributions of $\log_{10}$ MoM values of the biomarkers in unaffected pregnancies and in those that experienced preeclampsia are shown in Tables 2 and 3. The MoM values in the preeclampsia group and the fitted regression relationships with gestational age at delivery are shown in Figure 1. All markers showed more separation at earlier, rather than later, gestations; this is reflected in their superior performance at detection of early, rather than late, preeclampsia. It is notable that the regression lines for uterine artery PI and PAPP-A intersect 1 MoM close to term. These markers show little or no discriminatory power beyond approximately 40 weeks gestation, and they perform relatively poorly in screening for late preeclampsia. Conversely, MAP shows a degree of separation from 1 MoM at term, and the performance of MAP for term preeclampsia is relatively good.

FIGURE 2
**Receiver operating characteristic curves**




Data show the prediction of (**left**) preterm preeclampsia and (**right**) term preeclampsia by maternal factors (*black*) and combination of maternal factors with uterine artery pulsatility index (*blue*), mean arterial pressure (*green*), serum pregnancy associated plasma protein-A (*purple*), and placental growth factor (*red*).

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### Performance of screening for preeclampsia

The areas under the receiver operating characteristic curves and performance of screening for preeclampsia by maternal factors and biomarkers are given in Figure 2 and Tables 4 and 5. The performance of each biomarker in combination with maternal factors was superior to that of screening by maternal factors alone. Similarly, the performance by a combination of ≥2 biomarkers was superior to that of screening by individual biomarkers. The only exception was serum PAPP-A, which did not provide significant improvement to any combination of biomarkers that included serum PLGF. Starting with the full model, incorporating maternal factors with all 4 biomarkers and applying backward elimination resulted in the removal of PAPP-A at the first step for both preterm-preeclampsia ($P = .15$) and term-preeclampsia ($P = .98$). In the backward elimination, after the removal of PAPP-A, all other variables made significant contributions ($P < .05$). There is evidence therefore of a benefit for screening with the combination of maternal factors, uterine artery PI, MAP, and serum PLGF (triple test), but not for the inclusion of PAPP-A.

The performance of screening for preterm-preeclampsia and term-preeclampsia by the mini-combined test (MAP and PAPP-A), biophysical test (uterine artery PI and MAP), biochemical test (serum PLGF and PAPP-A) and the triple test (uterine artery PI, MAP and serum PLGF) is shown in Figure 3.

**TABLE 4**
**Detection rate at false-positive rates of 5% and 10% of preeclampsia with delivery at <37 and ≥37 weeks gestation in screening by maternal factors, biomarkers, and their combination**

| | Preeclampsia | | | | | |
|---|---|---|---|---|---|---|
| | <37 Weeks gestation | | | ≥37 Weeks gestation | | |
| | | False-positive detection rate (95% confidence interval) | | | False-positive detection rate (95% confidence interval) | |
| Method of screening | Area under the curve | 5% | 10% | Area under the curve | 5% | 10% |
| Maternal factors | 0.800 | 36 (30–41) | 49 (43–55) | 0.745 | 28 (24–31) | 38 (34–41) |
| Maternal factors plus | | | | | | |
| Mean arterial pressure | 0.845 | 44 (38–50) | 59 (53–65) | 0.781 | 30 (27–34) | 43 (40–47) |
| Uterine artery pulsatility index | 0.841 | 46 (40–52) | 60 (54–66) | 0.749 | 29 (26–33) | 39 (35–43) |
| Pregnancy-associated plasma protein-A | 0.822 | 40 (34–46) | 53 (48–59) | 0.748 | 28 (25–31) | 39 (35–42) |
| Placental growth factor | 0.872 | 50 (44–56) | 65 (60–71) | 0.764 | 29 (25–32) | 42 (38–45) |
| Mean arterial pressure, uterine artery pulsatility index | 0.876 | 53 (47–59) | 70 (64–75) | 0.785 | 32 (28–35) | 44 (41–48) |
| Mean arterial pressure, pregnancy-associated plasma protein-A | 0.860 | 48 (42–54) | 61 (55–66) | 0.783 | 31 (28–35) | 45 (41–49) |
| Mean arterial pressure, placental growth factor | 0.896 | 59 (53–64) | 73 (67–78) | 0.794 | 32 (29–36) | 47 (44–51) |
| Uterine artery pulsatility index, pregnancy-associated plasma protein-A | 0.851 | 48 (42–54) | 60 (54–65) | 0.751 | 29 (26–32) | 40 (36–43) |
| Uterine artery pulsatility index, placental growth factor | 0.884 | 58 (52–63) | 70 (64–75) | 0.766 | 30 (26–33) | 42 (38–46) |
| Placental growth factor, pregnancy-associated plasma protein-A | 0.873 | 50 (44–56) | 66 (60–71) | 0.764 | 29 (25–32) | 42 (38–46) |
| Mean arterial pressure, uterine artery pulsatility index, pregnancy-associated plasma protein-A | 0.884 | 55 (49–61) | 70 (65–75) | 0.787 | 32 (29–35) | 45 (41–48) |
| Mean arterial pressure, pregnancy-associated plasma protein-A, placental growth factor | 0.897 | 59 (53–65) | 73 (67–78) | 0.794 | 32 (29–36) | 48 (44–51) |
| Mean arterial pressure, uterine artery pulsatility index, placental growth factor | 0.906 | 65 (59–71) | 75 (70–80) | 0.796 | 33 (30–37) | 47 (44–51) |
| Uterine artery pulsatility index, pregnancy-associated plasma protein-A, placental growth factor | 0.885 | 57 (51–63) | 69 (64–74) | 0.766 | 30 (26–33) | 43 (39–46) |
| Mean arterial pressure, uterine artery pulsatility index, pregnancy-associated plasma protein-A, placental growth factor | 0.907 | 64 (58–70) | 75 (70–80) | 0.796 | 33 (29–36) | 48 (44–52) |

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**TABLE 5**
**Detection rate at false-positive rates of 5% and 10% of preeclampsia with delivery at <32, 32-36.9, 37-39.9, and ≥40 weeks gestation in screening by maternal factors, biomarkers, and their combination**

| | False-positive rate (95% confidence interval) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 5% | | | | | 10% | | | | |
| Method of screening | <32 Wk | $32^{+0}-36^{+6}$ Wk | $37^{+0}-39^{+6}$ Wk | ≥40 Wk | | <32 Wk | $32^{+0}-36^{+6}$ Wk | $37^{+0}-39^{+6}$ Wk | ≥40 Wk | |
| Maternal factors | 42 (30–55) | 34 (27–40) | 31 (27–35) | 21 (16–26) | | 53 (40–65) | 48 (42–55) | 41 (37–45) | 30 (25–36) | |
| Maternal factors plus | | | | | | | | | | |
| Mean arterial pressure | 48 (36–61) | 43 (36–50) | 35 (31–39) | 21 (17–27) | | 65 (52–76) | 58 (51–64) | 48 (44–52) | 34 (28–40) | |
| Uterine artery pulsatility index | 55 (42–67) | 43 (37–50) | 33 (29–37) | 21 (16–27) | | 65 (52–76) | 58 (52–65) | 43 (38–47) | 31 (26–37) | |
| Pregnancy-associated plasma protein-A | 44 (32–57) | 39 (33–46) | 32 (28–36) | 20 (15–25) | | 59 (46–71) | 52 (45–58) | 42 (38–47) | 31 (25–37) | |
| Placental growth factor | 67 (54–78) | 46 (39–52) | 33 (29–38) | 19 (15–25) | | 80 (69–89) | 61 (54–67) | 45 (41–50) | 35 (29–42) | |
| Mean arterial pressure, uterine artery pulsatility index | 56 (43–68) | 52 (45–58) | 36 (32–41) | 23 (18–29) | | 80 (69–89) | 67 (60–73) | 49 (44–53) | 36 (30–42) | |
| Mean arterial pressure, pregnancy-associated plasma protein-A | 52 (39–64) | 47 (40–54) | 36 (32–40) | 22 (17–28) | | 65 (52–76) | 59 (53–66) | 50 (45–54) | 36 (30–42) | |
| Mean arterial pressure, placental growth factor | 76 (64–85) | 54 (47–60) | 36 (32–40) | 26 (21–32) | | 83 (72–91) | 70 (63–76) | 53 (48–57) | 37 (31–44) | |
| Uterine artery pulsatility index, pregnancy-associated plasma protein-A | 59 (46–71) | 44 (38–51) | 33 (29–37) | 21 (16–27) | | 68 (56–79) | 57 (50–64) | 43 (39–48) | 33 (27–39) | |
| Uterine artery pulsatility index, placental growth factor | 74 (62–84) | 53 (46–59) | 34 (30–39) | 21 (16–26) | | 83 (72–91) | 65 (59–72) | 46 (41–50) | 35 (29–41) | |
| Placental growth factor, pregnancy-associated plasma protein-A | 67 (54–78) | 45 (39–52) | 33 (29–37) | 20 (15–26) | | 79 (67–88) | 62 (55–68) | 46 (41–50) | 36 (30–42) | |
| Mean arterial pressure, uterine artery pulsatility index, pregnancy-associated plasma protein-A | 65 (52–76) | 52 (45–59) | 36 (31–40) | 25 (19–30) | | 83 (72–91) | 66 (60–73) | 50 (45–54) | 35 (29–41) | |
| Mean arterial pressure, pregnancy-associated plasma protein-A, placental growth factor | 76 (64–85) | 54 (47–61) | 36 (32–40) | 26 (21–32) | | 85 (74–92) | 69 (63–75) | 53 (48–57) | 39 (33–45) | |
| Mean arterial pressure, uterine artery pulsatility index, placental growth factor | 82 (70–90) | 60 (53–67) | 37 (33–42) | 27 (21–32) | | 89 (79–96) | 71 (64–77) | 53 (48–57) | 38 (32–44) | |
| Uterine artery pulsatility index, pregnancy-associated plasma protein-A, placental growth factor | 76 (64–85) | 52 (45–58) | 34 (30–38) | 21 (16–26) | | 83 (72–91) | 65 (58–71) | 46 (42–51) | 36 (30–42) | |
| Mean arterial pressure, uterine artery pulsatility index, pregnancy-associated plasma protein-A, placental growth factor | 82 (70–90) | 59 (52–65) | 37 (33–41) | 26 (21–32) | | 89 (79–96) | 71 (64–77) | 54 (49–58) | 38 (32–44) | |

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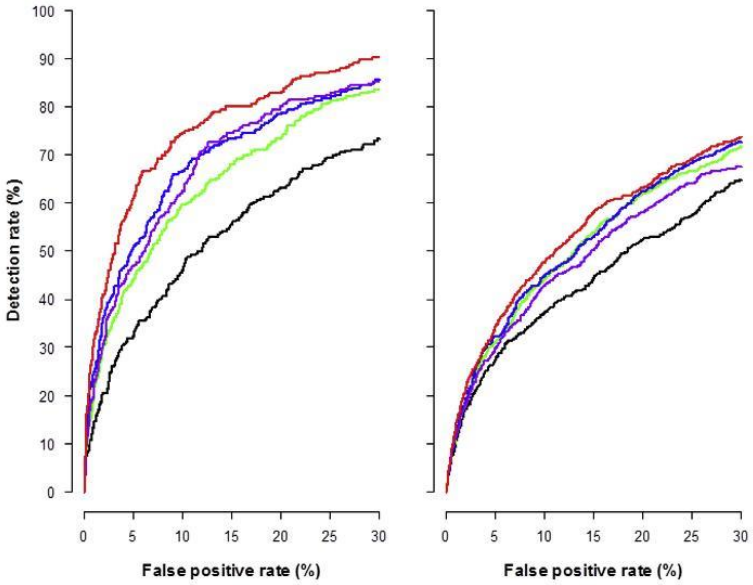
FIGURE 3
**Receiver operating characteristic curves**




Data show the prediction of (**left**) preterm preeclampsia and (**right**) term preeclampsia by maternal factors (*black*), mini-combined test (*green*), biophysical test (*blue*), biochemical test (*purple*), and the triple test (*red*).

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### Performance of screening for preeclampsia in subgroups

The performance of screening by the triple test in the prediction of preterm-preeclampsia and term-preeclampsia for nulliparous and parous women of Afro-Caribbean and white racial origin are given in Tables 6 and 7. In these calculations, a risk cut-off was selected to achieve a screen-positive rate of approximately 10%. At a risk cut-off of 1 in 70 for preterm-preeclampsia and 1 in 15 for term-preeclampsia, the FPR and detection rate (DR) were higher in nulliparous than in parous women, in parous women with, rather than without, preeclampsia in a previous pregnancy, and in women of Afro-Caribbean, rather than white, racial origin. In all groups, the risk of being affected, given a screen-positive result, was considerably higher that the prevalence of the disease; whereas in those with a screen negative result, the risk was considerably reduced.

In the lowest-risk group, white parous women with no history of preeclampsia, which comprised 35% of the population (12,726/35,948) and accounted for 18% of cases (52/292) of preterm-preeclampsia, the DR for preterm-preeclampsia was 50%, and the FPR was 2.7%; in total, 489 tests would need to be performed for each true positive identified. In the highest-risk group, Afro-Caribbean women with a history of preeclampsia, which comprised 1% of the population (370/35,948) and accounted for 7.5% of the cases (22/292) of preterm-preeclampsia, the DR for preterm-preeclampsia was 100%, and the FPR was 63.4%; in total 17 tests would need to be performed for each true positive that is identified.

The algorithm for estimation of risk for preeclampsia based on maternal characteristics and biomarkers will be available free-of charge in the website of the Fetal Medicine Foundation (www.fetalmedicine.com).

## Comment

### Principal findings of this study

In pregnancies that experience preeclampsia, the MoM values of uterine artery PI and MAP are increased, and the values of serum PAPP-A and PLGF are decreased. For all biomarkers, the deviation from normal is greater for early, rather than late, preeclampsia; therefore, the performance of screening is related inversely to the gestational age at which delivery becomes necessary for maternal and/or fetal indications.

Screening for preeclampsia by a combination of maternal factors, uterine artery PI, MAP, and serum PLGF at 11-13 weeks gestation can predict 75% of preterm-preeclampsia and 47% of term-preeclampsia, at an FPR of 10%. Such DRs are superior to the respective values of 49% and 38% that are achieved by screening with maternal factors alone. The performance of screening by both biophysical and biochemical markers is superior to screening by either method alone. Although serum PAPP-A improves the performance of screening by maternal factors or biophysical markers, we found no evidence of improvement to any combination of biomarkers that include serum PLGF.

The study has highlighted that, in screening for preeclampsia, the FPR and DR are influenced by the characteristics of the study population; for a given risk cut-off, they are both higher in nulliparous rather than in parous women and in those of Afro-Caribbean rather than white racial origin. Consequently, comparison of the performance of screening between studies requires the appropriate adjustments for the characteristics of the population under investigation. Although the risk of preeclampsia is higher in nulliparous than parous women, the contribution of the latter group to preeclampsia should not be underestimated because approximately 45% of cases of preterm-preeclampsia were from parous women, which included 16% from parous women with preeclampsia in a previous pregnancy and 29% from parous women without a history of preeclampsia. Similarly, 40% of cases of term-preeclampsia were from parous women, which included 14%

**TABLE 6**
**Performance of screening for preeclampsia with delivery at <37 weeks gestation by an algorithm that combined maternal factors, uterine artery pulsatility index, mean arterial pressure, and serum placental growth factor at a risk cut-off of 1 in 70**

| Group | Prevalence, % (95% confidence interval) | Screen-positive rate, % (95% confidence interval) | False-positive rate, % (95% confidence interval) | Detection rate, % (95% confidence interval) | Risk of being affected given the result | |
|---|---|---|---|---|---|---|
| | | | | | Screen-positive, % (95% confidence interval)[a] | Screen-negative, % (95% confidence interval)[b] |
| All pregnancies | 0.81 (0.72–0.91) | 11.3 (11.0–11.6) | 9.4 (9.1–9.7) | 75.3 (70.0–80.2) | 5.4 (4.8–6.2) | 0.23 (0.18–0.28) |
| Nulliparous | 0.92 (0.78–1.08) | 14.1 (13.6–14.6) | 12.1 (11.6–12.6) | 75.00 (67.6–81.5) | 4.9 (4.1–5.9) | 0.27 (0.19–0.36) |
| Parous | 0.71 (0.59–0.84) | 8.7 (8.3–9.1) | 7.0 (6.6–7.4) | 75.8 (67.5–82.8) | 6.2 (5.1–7.5) | 0.19 (0.13–0.27) |
| No previous preeclampsia | 0.49 (0.39–0.61) | 5.3 (5.0–5.7) | 4.5 (4.2–4.8) | 64.7 (53.6–74.8) | 6.0 (4.5–7.7) | 0.18 (0.12–0.26) |
| Previous preeclampsia | 3.68 (2.72–4.87) | 54.0 (51.2–56.8) | 48.5 (45.4–51.7) | 95.7 (85.5–99.5) | 6.5 (4.8–8.6) | 0.34 (0.04–1.23) |
| Afro-Caribbean | 1.84 (1.53–2.19) | 27.3 (26.2–28.3) | 23.2 (22.2–24.3) | 87.8 (80.7–93.0) | 5.9 (4.9–7.1) | 0.31 (0.17–0.51) |
| Nulliparous | 2.14 (1.61–2.78) | 37.6 (35.7–39.6) | 33.6 (31.6–35.6) | 87.0 (75.1–94.6) | 4.9 (3.7–6.5) | 0.44 (0.18–0.91) |
| Parous | 1.66 (1.29–2.10) | 20.9 (19.7–22.2) | 17.2 (16.0–18.5) | 88.4 (78.4–94.9) | 7.0 (5.4–8.9) | 0.24 (0.11–0.48) |
| No previous preeclampsia | 1.24 (0.91–1.65) | 15.6 (14.5–16.8) | 13.1 (12.0–14.3) | 83.0 (69.2–92.4) | 6.6 (4.7–8.9) | 0.25 (0.11–0.49) |
| Previous preeclampsia | 5.95 (3.76–8.86) | 75.4 (70.7–79.7) | 70.3 (64.5–75.7) | 100 (84.6–100) | 7.9 (5.0–11.7) | 0.00 (0.00–3.97) |
| White | 0.54 (0.45–0.63) | 7.2 (6.9–7.5) | 6.0 (5.7–6.3) | 62.6 (54.0–70.6) | 4.7 (3.8–5.8) | 0.22 (0.16–0.28) |
| Nulliparous | 0.66 (0.53–0.82) | 9.5 (9.0–1.0) | 8.2 (7.7–8.7) | 65.5 (54.6–75.4) | 4.6 (3.5–5.9) | 0.25 (0.17–0.36) |
| Parous | 0.41 (0.31–0.54) | 4.6 (4.4–5.1) | 3.9 (3.5–4.2) | 57.7 (43.2–71.3) | 5.0 (3.4–7.0) | 0.18 (0.11–0.27) |
| No previous preeclampsia | 0.29 (0.20–0.40) | 2.1 (1.9–2.4) | 1.9 (1.6–2.1) | 38.2 (22.2–56.4) | 5.1 (2.7–8.6) | 0.18 (0.11–0.28) |
| Previous preeclampsia | 2.23 (1.33–3.51) | 43.4 (40.0–46.9) | 38.7 (35.0–42.5) | 94.4 (72.7–99.9) | 4.9 (2.9–7.7) | 0.22 (0.01–1.22) |

[a] Same as positive predictive value; [b] Same as 1 – negative predictive value.
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**TABLE 7**
**Performance of screening for preeclampsia with delivery at ≥37 weeks gestation by an algorithm that combined maternal factors—uterine artery pulsatility index—mean arterial pressure and serum placental growth factor at a risk cut-off of 1 in 15**

| Group | Prevalence, % (95% confidence interval) | Screen-positive rate, % (95% confidence interval) | False-positive rate, % (95% confidence interval) | Detection rate, % (95% confidence interval) | Risk of being affected given the result | |
|---|---|---|---|---|---|---|
| | | | | | Screen-positive, % (95% confidence interval)[a] | Screen-negative, % (95% confidence interval)[b] |
| All pregnancies | 2.25 (2.10–2.42) | 10.4 (10.1–10.7) | 8.9 (8.6–9.2) | 46.6 (43.0–50.2) | 10.1 (9.2–11.2) | 1.34 (1.22–1.48) |
| Nulliparous | 2.82 (2.57–3.08) | 13.0 (12.5–13.5) | 11.4 (11.0–12.0) | 41.8 (37.2–46.4) | 9.1 (7.9–10.4) | 1.89 (1.67–2.12) |
| Parous | 1.73 (1.54–1.93) | 8.0 (7.6–8.4) | 6.6 (6.2–7.0) | 54.0 (48.2–59.7) | 11.7 (10.1–13.5) | 0.86 (0.73–1.02) |
| No previous preeclampsia | 1.20 (1.04–1.38) | 4.3 (4.0–4.6) | 3.7 (3.4–4.0) | 34.3 (27.8–41.4) | 9.7 (7.6–12.1) | 0.82 (0.69–0.98) |
| Previous preeclampsia | 9.28 (7.66–11.12) | 61.1 (58.2–64.0) | 56.5 (53.2–59.7) | 90.6 (83.3–95.4) | 13.8 (11.3–16.5) | 2.25 (1.09–4.10) |
| Afro-Caribbean | 4.36 (3.86–4.90) | 28.8 (27.7–30.0) | 25.6 (24.5–26.7) | 74.2 (68.5–79.3) | 11.2 (9.8–12.8) | 1.58 (1.24–1.99) |
| Nulliparous | 5.52 (4.63–6.52) | 44.2 (42.2–46.3) | 41.1 (39.0–43.3) | 79.1 (71.0–85.7) | 9.9 (8.1–11.9) | 2.07 (1.37–3.00) |
| Parous | 3.66 (3.09–4.30) | 19.5 (18.3–20.8) | 16.6 (15.4–17.9) | 69.7 (61.5–77.1) | 13.1 (10.7–15.7) | 1.38 (1.00–1.85) |
| No previous preeclampsia | 2.64 (2.14–3.22) | 13.5 (12.4–14.7) | 11.8 (10.7–12.9) | 55.3 (44.7–65.6) | 10.8 (8.2–13.9) | 1.36 (0.99–1.84) |
| Previous preeclampsia | 15.0 (11.27–19.39) | 86.3 (82.0–89.8) | 83.0 (77.7–87.5) | 97.9 (88.9–100) | 17.0 (12.8–22.0) | 2.27 (0.06–12.02) |
| White | 1.73 (1.57–1.90) | 5.9 (5.6–6.2) | 5.0 (4.7–5.3) | 30.4 (26.0–35.0) | 8.9 (7.5–10.5) | 1.28 (1.14–1.43) |
| Nulliparous | 2.30 (2.05–2.58) | 7.3 (6.8–7.8) | 6.4 (5.9–6.8) | 26.5 (21.5–32.0) | 8.4 (6.7–10.4) | 1.83 (1.59–2.09) |
| Parous | 1.14 (0.96–1.34) | 4.4 (4.1–4.8) | 3.6 (3.3–4.0) | 38.4 (30.3–47.1) | 9.9 (7.5–12.7) | 0.73 (0.59–0.91) |
| No previous preeclampsia | 0.78 (0.63–0.96) | 1.5 (1.3–1.7) | 1.3 (1.1–1.5) | 13.5 (7.2–22.4) | 7.2 (3.8–12.2) | 0.69 (0.54–0.86) |
| Previous preeclampsia | 6.66 (4.97–8.71) | 50.3 (46.6–53.9) | 45.8 (41.8–49.8) | 83.7 (70.3–92.7) | 11.1 (8.1–14.7) | 2.19 (0.95–4.26) |

[a] Same as positive predictive value; [b] Same as 1 – negative predictive value.
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from parous women with preeclampsia in a previous pregnancy and 26% from parous women without a history of preeclampsia. In all groups, after combined screening, the risk of being affected, given a screen-positive result, was considerably increased; if the screen result was negative, the risk was considerably reduced.

## Strengths and limitations

The strengths of this first-trimester screening study for preeclampsia are (1) examination of a large population of pregnant women who attended for routine care in a gestational age range that is used widely for the assessment of risk for chromosomal abnormalities, (2) the recording of data on maternal characteristics and medical history to identify known risk factors that are associated with preeclampsia, (3) use of a specific method and appropriately trained doctors to measure uterine artery PI and MAP, (4) the use of automated machines to provide accurate measurement within 40 minutes of sampling of maternal serum concentration of metabolites that have been shown to be altered in pregnancies and to be associated with impaired placentation, (5) expression of the values of the biomarkers as MoMs after adjustment for factors that affect the measurements, and (6) the use of Bayes theorem to combine the previous risk from maternal factors with biomarkers to estimate patient-specific risks and the performance of screening for preeclampsia delivery at different stages of pregnancy.

A limitation of the study is that the performance of screening by a model that was derived and tested with the use of the same dataset is overestimated. We have used cross validation to reduce this effect, but we acknowledge that this approach fails to capture the overestimation of performance because of model selection. Consequently, external validation on independent data from different sources is required.

## Comparison with previous studies

Several studies have documented that development of preeclampsia is associated with a first-trimester increase in uterine artery PI and MAP and a decrease in serum PLGF and PAPP-A.[7-10,22-24] In previous studies, we proposed a model of screening for preeclampsia based on Bayes theorem to combine the a priori risk from maternal factors with biomarkers.[8,9] In this study, we prospectively examined a large population of pregnancies in which all 4 biomarkers were measured and conducted a 5-fold cross validation study to assess the performance of that screening.

## Clinical implications of the study

Screening and diagnosis of preeclampsia traditionally is based on the demonstration of elevated blood pressure and proteinuria during a routine clinical visit in the late second- or third-trimester of pregnancy. In a proposed new pyramid of pregnancy care,[25] an integrated clinic at 11-13 weeks gestation, in which biophysical and biochemical markers are combined with maternal factors, aims to identify pregnancies that are at high risk of experiencing preeclampsia and, through pharmacologic intervention (with such medications as low-dose aspirin), to reduce the prevalence of these complications.[3,4] In pregnancies with impaired placentation, the use of low-dose aspirin at >16 weeks gestation does not prevent the subsequent development of preeclampsia.[3,4]

Our finding that the performance of first-trimester screening is better for preterm-preeclampsia rather than term-preeclampsia is particularly important because the incidence of adverse fetal and maternal short-term and long-term consequences of preeclampsia are related inversely to the gestational age at onset of the disease[26-31] and the prophylactic use of low-dose aspirin is effective in the prevention of preterm-preeclampsia rather than term-preeclampsia.[4]

There are various levels of complexity and implications in terms of general applicability and costs for the various components of the combined test, compared with screening by maternal factors alone. Measurement of MAP can be undertaken by health care assistants after minimal training, with the use of inexpensive equipment, and takes a few minutes to perform. Measurement of serum PAPP-A and quality assurance for such measurement are already in place in centers that provide routine first-trimester combined screening for Down syndrome. Measurement of serum PLGF can be undertaken on the same sample and by the same machines as for PAPP-A, but at an additional cost. Measurement of uterine artery PI can be undertaken within a few minutes by the same sonographers and machines as part of the current 11-13 week scan, which is used widely in screening for Down syndrome; however, the sonographers will require specific training for this measurement and quality assurance of their results. Consequently, the choice of test for screening ultimately will depend not only on the basis of performance but also on the feasibility of implementation and health economic considerations. In terms of performance, the DR of preterm-preeclampsia at FPR of 10% is approximately 50% in screening by maternal factors alone and 60%, 65%, 70%, and 75% in screening by the mini-combined test, the biochemical test, the biophysical test, and the triple combined test, respectively. ■

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# Accuracy of competing-risks model in screening for pre-eclampsia by maternal factors and biomarkers at 11–13 weeks' gestation

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**KEYWORDS:** Bayes' theorem; first-trimester screening; mean arterial pressure; placental growth factor; pre-eclampsia; pregnancy-associated plasma protein-A; pyramid of pregnancy care; survival model; uterine artery Doppler

## ABSTRACT

***Objective*** *To examine the diagnostic accuracy of a previously developed model for prediction of pre-eclampsia (PE) by a combination of maternal factors and biomarkers at 11–13 weeks' gestation.*

***Methods*** *This was a prospective first-trimester multicenter study of screening for PE in 8775 singleton pregnancies. A previously published algorithm was used for the calculation of patient-specific risk of PE in each individual. The detection rates (DRs) and false-positive rates (FPRs) for delivery with PE <32, <37 and ≥37 weeks were estimated and compared with those for the dataset used for development of the algorithm.*

***Results*** *In the study population, 239 (2.7%) cases developed PE, of which 17 (0.2%), 59 (0.7%) and 180 (2.1%) developed PE <32, <37 and ≥37 weeks, respectively. With combined screening by maternal factors, mean arterial pressure, uterine artery pulsatility index and serum placental growth factor, the DR was 100% (95% CI, 80–100%) for PE <32 weeks, 75% (95% CI, 62–85%) for PE <37 weeks and 43% (95% CI, 35–50%) for PE ≥37 weeks, at a 10% FPR. These DRs were similar to the estimated rates for the dataset used for development of the model: 89% (95% CI, 79–96%) for PE <32 weeks, 75% (95% CI, 70–80%) for PE <37 weeks and 47% (95% CI, 44–51%) for PE ≥37 weeks.*

***Conclusion*** *Assessment of a combination of maternal factors and biomarkers at 11–13 weeks provides effective first-trimester screening for preterm PE. Copyright © 2017 ISUOG. Published by John Wiley & Sons Ltd.*

## INTRODUCTION

Effective screening for preterm pre-eclampsia (PE) can be provided at 11–13 weeks' gestation by assessment of a combination of maternal characteristics and medical history (maternal factors) with multiples of the median (MoM) values of mean arterial pressure (MAP), uterine artery pulsatility index (UtA-PI) and serum placental growth factor (PlGF) and pregnancy-associated plasma protein-A (PAPP-A). In a previous study, we used data from prospective screening in 35 948 singleton pregnancies at 11–13 weeks to develop an algorithm for the calculation of patient-specific risk of PE[1]. Bayes' theorem was used to combine the *a-priori* risk from maternal factors[2] with various combinations of MAP, UtA-PI, PAPP-A and PlGF[1]. In pregnancies with PE, the deviation from normal for each biomarker was inversely related to the gestational age at delivery and, consequently, the performance of screening was better for early than late PE. The performance of each biomarker in combination with maternal factors was superior to that of screening by maternal factors alone. Similarly, the performance of screening by a combination of two or more biomarkers was superior to that by

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individual biomarkers. The only exception was serum PAPP-A, which did not provide significant improvement to any combination of biomarkers that included serum PlGF. With combined screening by maternal factors, MAP, UtA-PI and PlGF, the detection rate (DR) of delivery with PE < 32, < 37 and ≥ 37 weeks was 89%, 75% and 47%, respectively, at a false-positive rate (FPR) of 10%[1]. A limitation of the study is that the performance of screening by a model derived and tested using the same dataset may be overestimated.

The objective of this study was to determine the accuracy of the previously reported first-trimester screening model for PE[1] in a prospective, non-intervention, multicenter study including 8775 singleton pregnancies. We hypothesize that the performance of screening would be similar to that estimated from the original model[1].

## METHODS

### Study design and participants

This was a prospective, non-intervention, multicenter study in singleton pregnancies at 11 + 0 to 13 + 6 weeks' gestation in women booking for routine pregnancy care at: King's College Hospital, London, UK; Medway Maritime Hospital, Gillingham, UK; Homerton University Hospital, London, UK; North Middlesex University Hospital, London, UK; Southend University Hospital, Essex, UK; Lewisham University Hospital, London, UK; Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain; Hospital Universitario San Cecilio, Granada, Spain; Hospiten Sur, Tenerife, Spain; Centre Hospitalier Universitaire Brugmann, Brussels, Belgium; Attikon University Hospital, Athens, Greece; and Ospedale Maggiore Policlinico, Milan, Italy. The women were screened between February and September 2015 and gave written informed consent to participate in the study, which was approved by the National Health Service Research Ethics Committee in the UK and the Ethics Committee of each participating hospital in other countries. The Standards for Reporting Diagnostic Accuracy Studies (STARD)[3] was adhered to.

Eligibility criteria for study inclusion were maternal age ≥ 18 years, no serious mental illness or learning difficulty, singleton pregnancy with live fetus demonstrated on 11–13-week ultrasound scan and subsequent delivery of a phenotypically normal live birth or stillbirth ≥ 24 weeks' gestation. Multiple pregnancies, those with aneuploidy or major fetal abnormality and those ending in termination or miscarriage were excluded.

### Test methods

The index test, or the test for which the accuracy was being evaluated, was the previously reported algorithm for first-trimester assessment of risk for PE by maternal factors and various combinations of MAP, UtA-PI, PAPP-A and PlGF[1]. Maternal factors were recorded[2] and MAP was measured by a validated automated device following a standardized protocol[4]. Transabdominal color Doppler ultrasound was performed to measure the left and right UtA-PI and the average value was recorded[5]. Serum PAPP-A and PlGF concentrations were measured using an automated device (PAPP-A and PlGF 1-2-3™ kits, DELFIA® Xpress random access platform; PerkinElmer Inc., Wallac Oy, Turku, Finland). All operators performing Doppler assessment had received the appropriate Certificate of Competence from The Fetal Medicine Foundation. Measured values of MAP, UtA-PI, PAPP-A and PlGF were expressed as MoM, adjusting for those characteristics found to provide a substantive contribution to the $\log_{10}$-transformed value, including maternal factors, in the prior model[6–9].

The index test was carried out prospectively in consecutive singleton pregnancies at 11 + 0 to 13 + 6 weeks' gestation; gestational age was determined from the measurement of fetal crown–rump length[10]. The results from screening were not made available to the patients or their physicians.

The target condition was PE, as defined by the International Society for the Study of Hypertension in Pregnancy[11]. PE was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg on at least two occasions 4 hours apart, developing after 20 weeks of gestation in previously normotensive women. Hypertension was defined as proteinuria of ≥ 300 mg in 24 h or two readings of at least ++ on dipstick analysis of midstream or catheter urine specimens if no 24-h collection was available. PE superimposed on chronic hypertension was defined as significant proteinuria (as defined above) developing after 20 weeks of gestation in women with known chronic hypertension (history of hypertension before conception or presence of hypertension at booking visit before 20 weeks' gestation, in the absence of trophoblastic disease).

Data on pregnancy outcome were collected from the hospital maternity records of the women. The obstetric records of all women with pre-existing or pregnancy-associated hypertension were examined to determine if the condition was PE.

### Statistical analysis

The previously described algorithm[1] was used for calculation of patient-specific risk of delivery with PE < 32, < 37 and ≥ 37 weeks' gestation. The prespecified analyses for performance of screening by maternal factors and any combinations of maternal factors with MAP, UtA-PI, PAPP-A and PlGF were estimation of areas under the receiver–operating characteristics curve (AUC) and DR, with 95% CI, at FPRs of 5% and 10%. The statistical software package R was used for data analyses[12].

## RESULTS

### Participants

During the study period, 9041 pregnancies met the inclusion criteria and underwent screening for PE. A

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**Table 1** Characteristics of women with normal singleton pregnancy and of those who developed pre-eclampsia (PE) with delivery < 32 weeks, < 37 weeks or ≥ 37 weeks

| | | *PE with delivery at:* | | |
|---|---|---|---|---|
| *Characteristic* | *Normal (n = 8536)* | *< 32 weeks (n = 17)* | *< 37 weeks (n = 59)* | *≥ 37 weeks (n = 180)* |
| Maternal age (years) | 31.5 (27.3–35.0) | 29.8 (26.7–34.6) | 30.6 (26.0–34.7) | 31.2 (27.8–34.8) |
| Maternal weight (kg) | 66.2 (58.8–76.9) | 72.6 (65.6–86.0) | 69.8 (63.0–87.8) | 75.0 (64.9–84.0) |
| Maternal height (cm) | 165 (160–169) | 164 (161–166) | 164 (160–169) | 164 (159–168) |
| Body mass index (kg/m$^2$) | 24.5 (21.9–28.2) | 27.3 (23.9–31.8) | 27.1 (23.6–31.8) | 27.8 (23.9–31.5) |
| Gestational age (weeks) | 12.7 (12.3–13.1) | 12.6 (12.3–12.7) | 12.7 (12.4–13.0) | 12.7 (12.3–13.2) |
| Racial origin | | | | |
| Caucasian | 6716 (78.7) | 8 (47.1) | 38 (64.4) | 129 (71.7) |
| Afro-Caribbean | 1040 (12.2) | 8 (47.1) | 14 (23.7) | 36 (20.0) |
| East Asian | 153 (1.8) | 0 (0.0) | 0 (0.0) | 1 (0.6) |
| South Asian | 447 (5.2) | 0 (0.0) | 3 (5.1) | 12 (6.7) |
| Mixed | 180 (2.1) | 1 (5.9) | 4 (6.8) | 2 (1.1) |
| Medical history | | | | |
| Chronic hypertension | 75 (0.9) | 3 (17.7) | 9 (15.3) | 16 (8.9) |
| Diabetes mellitus | 63 (0.7) | 2 (11.8) | 3 (5.1) | 2 (1.1) |
| APS/SLE | 32 (0.4) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Cigarette smoker | 717 (8.4) | 1 (5.9) | 4 (6.8) | 11 (6.1) |
| Family history of PE | 434 (5.1) | 1 (5.9) | 7 (11.9) | 17 (9.4) |
| Mode of conception | | | | |
| Spontaneous | 8254 (96.7) | 17 (100) | 57 (96.6) | 173 (96.1) |
| *In-vitro* fertilization | 218 (2.6) | 0 (0.0) | 2 (3.4) | 7 (3.9) |
| Ovulation drugs | 64 (0.7) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Parity | | | | |
| Nulliparous | 3972 (46.5) | 11 (64.7) | 36 (61.0) | 119 (66.1) |
| Parous | | | | |
| No previous PE | 4396 (51.5) | 4 (23.5) | 17 (28.8) | 46 (25.6) |
| Previous PE | 168 (2.0) | 2 (11.8) | 6 (10.2) | 15 (8.3) |
| Interpregnancy interval (years) | 2.7 (1.6–4.6) | 5.4 (4.3–7.2) | 4.1 (2.4–6.8) | 3.4 (2.0–5.4) |

Data are given as median (interquartile range) or *n* (%). Comparisons between outcome groups were by chi-square or Fisher's exact tests for categorical variables and Mann–Whitney *U*-test for continuous variables. APS, antiphospholipid syndrome; SLE, systemic lupus erythematosus.

**Table 2** Performance of screening for delivery with pre-eclampsia (PE) < 32, < 37 or ≥ 37 weeks' gestation in validation dataset of 8775 singleton pregnancies using previously developed algorithm based on maternal factors and combinations of biomarkers

| | *PE with delivery < 32 weeks (n = 17)* | | | *PE with delivery < 37 weeks (n = 59)* | | | *PE with delivery ≥ 37 weeks (n = 180)* | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *DR (%) at:* | | | *DR (%) at:* | | | *DR (%) at:* | |
| *Screening method* | *AUC* | *FPR = 5%* | *FPR = 10%* | *AUC* | *FPR = 5%* | *FPR = 10%* | *AUC* | *FPR = 5%* | *FPR = 10%* |
| Maternal factors | 0.8045 | 41 (18–67) | 53 (28–77) | 0.7583 | 29 (18–42) | 41 (28–54) | 0.7449 | 18 (13–25) | 37 (30–45) |
| Maternal factors plus: | | | | | | | | | |
| MAP | 0.9071 | 59 (33–82) | 71 (44–90) | 0.8243 | 36 (24–49) | 47 (34–61) | 0.7789 | 26 (20–33) | 37 (30–45) |
| UtA-PI | 0.9309 | 71 (44–90) | 82 (57–96) | 0.8537 | 47 (34–61) | 61 (47–73) | 0.7539 | 22 (16–29) | 39 (32–47) |
| PAPP-A | 0.8546 | 47 (23–72) | 59 (33–82) | 0.7825 | 37 (25–51) | 47 (34–61) | 0.7504 | 21 (15–28) | 37 (30–44) |
| PlGF | 0.9506 | 65 (38–86) | 88 (64–99) | 0.8722 | 49 (36–63) | 63 (49–75) | 0.7578 | 20 (14–27) | 39 (32–46) |
| MAP, UtA-PI | 0.9667 | 82 (57–96) | 94 (71–100) | 0.8958 | 53 (39–66) | 71 (58–82) | 0.7875 | 27 (20–34) | 41 (34–49) |
| MAP, PAPP-A | 0.9133 | 65 (38–86) | 76 (50–93) | 0.8342 | 41 (28–54) | 49 (36–63) | 0.7827 | 28 (21–35) | 40 (33–48) |
| MAP, PlGF | 0.9674 | 76 (50–93) | 88 (64–99) | 0.8985 | 53 (39–66) | 69 (56–81) | 0.7870 | 29 (22–36) | 43 (36–51) |
| UtA-PI, PAPP-A | 0.9339 | 71 (44–90) | 82 (57–96) | 0.8583 | 49 (36–63) | 66 (53–78) | 0.7571 | 24 (18–31) | 40 (33–48) |
| UtA-PI, PlGF | 0.9772 | 82 (57–96) | 100 (80–100) | 0.9000 | 61 (47–73) | 75 (62–85) | 0.7619 | 22 (16–29) | 39 (32–47) |
| PlGF, PAPP-A | 0.9510 | 65 (38–86) | 88 (64–99) | 0.8741 | 51 (37–64) | 66 (53–78) | 0.7589 | 20 (14–27) | 39 (32–47) |
| MAP, UtA-PI, PAPP-A | 0.9644 | 88 (64–99) | 94 (71–100) | 0.8956 | 61 (47–73) | 69 (56–81) | 0.7892 | 29 (22–36) | 42 (35–50) |
| MAP, PAPP-A, PlGF | 0.9672 | 76 (50–93) | 88 (64–99) | 0.8998 | 54 (41–67) | 69 (56–81) | 0.7882 | 29 (22–36) | 43 (36–51) |
| MAP, UtA-PI, PlGF | 0.9870 | 94 (71–100) | 100 (80–100) | 0.9242 | 66 (53–78) | 75 (62–85) | 0.7916 | 32 (25–39) | 43 (35–50) |
| UtA-PI, PAPP-A, PlGF | 0.9769 | 82 (57–96) | 100 (80–100) | 0.9004 | 61 (47–73) | 75 (62–85) | 0.7626 | 23 (17–30) | 38 (31–46) |
| MAP, UtA-PI, PAPP-A, PlGF | 0.9865 | 94 (71–100) | 100 (80–100) | 0.9241 | 66 (53–78) | 80 (67–89) | 0.7923 | 31 (24–38) | 43 (35–50) |

Values in parentheses are 95% CI. AUC, area under receiver–operating characteristics curve; DR, detection rate; FPR, false-positive rate; MAP, mean arterial pressure; PAPP-A, pregnancy-associated plasma protein-A; PlGF, placental growth factor; UtA-PI, uterine artery pulsatility index.

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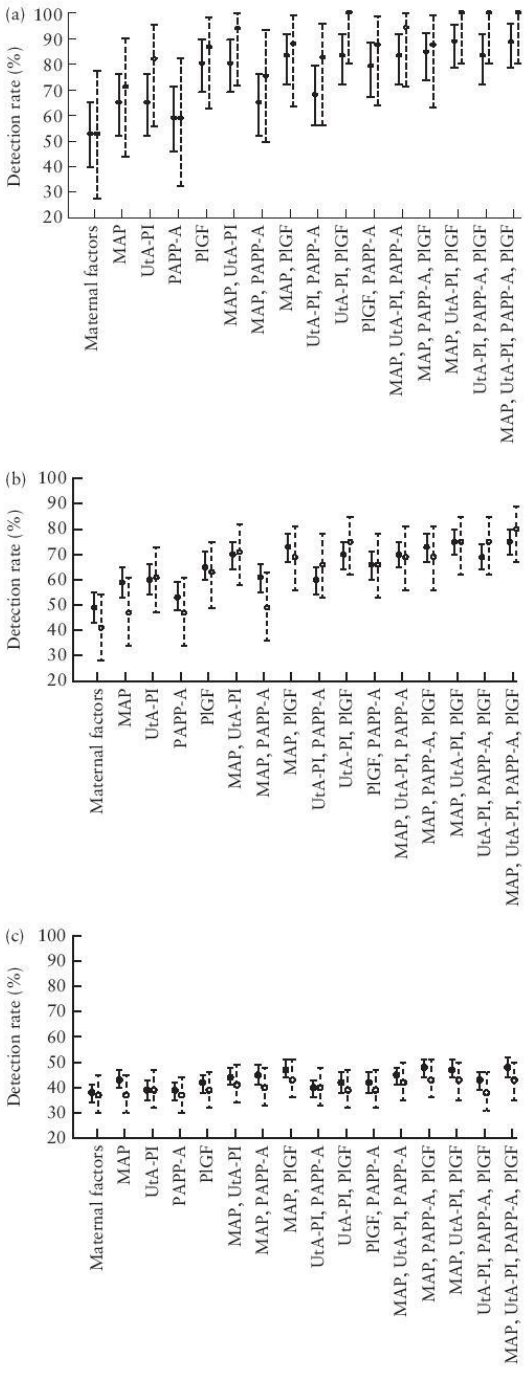


**Figure 1** Detection rate (95% CI), at 10% false-positive rate, of pre-eclampsia delivering at: (a) < 32 weeks; (b) < 37 weeks; or (c) ≥ 37 weeks of gestation, with screening by maternal factors and combinations of biomarkers in current dataset (○) and in dataset used for development of screening algorithm[1] (●). MAP, mean arterial pressure; PAPP-A, pregnancy-associated plasma protein-A; PlGF, placental growth factor; UtA-PI, uterine artery pulsatility index.

total of 266 (2.9%) cases were excluded because they had a major fetal defect ($n = 33$), the pregnancy resulted in termination ($n = 39$) or miscarriage ($n = 88$) or there was no follow up ($n = 106$).

In the study population of 8775 pregnancies, 239 (2.7%) cases developed PE, of which 17 (0.2%), 59 (0.7%) and 180 (2.1%) developed PE < 32, < 37 and ≥ 37 weeks, respectively. Baseline demographic and clinical characteristics of the participants are shown in Table 1. In total, 12 maternity hospitals in five different countries were involved in patient recruitment, 127 doctors participated in the measurement of UtA-PI and 152 doctors or nurses were involved in the measurement of MAP.

### Test results

The AUC and DR, at FPRs of 5% and 10%, of delivery with PE < 32, < 37 and ≥ 37 weeks' gestation with screening by maternal factors and biomarkers using the previously reported algorithm[1] are given in Table 2 and compared to previously reported values in Figure 1. The DRs in this validation dataset were similar to the estimated rates for the dataset used for development of the model.

The performance of screening for PE < 37 weeks was superior to that of PE ≥ 37 weeks. The best performance of screening was achieved by a combination of maternal factors, MAP, UtA-PI and PlGF and this was not improved significantly by addition of PAPP-A.

## DISCUSSION

### Main findings

This prospective multicenter validation study demonstrates the feasibility of incorporating first-trimester screening for PE into routine clinical practice. The findings demonstrate that the performance of screening for PE at 11–13 weeks by a combination of maternal factors and biomarkers is similar to that estimated from the original model[1]. The DR of screening by maternal factors, MAP, UtA-PI and PlGF, at 10% FPR, was 100% (95% CI, 80–100%) for PE < 32 weeks, 75% (95% CI, 62–85%) for PE < 37 weeks and 43% (95% CI, 35–50%) for PE ≥ 37 weeks; the estimated rates for the dataset used for development of the model were 89% (95% CI, 79–96%), 75% (95% CI, 70–80%) and 47% (95% CI, 44–51%), respectively[1].

### Study limitations

The main limitation of the study relates to the low incidence of delivery with PE, with the inevitable wide CIs obtained for performance of screening. Nevertheless, the values obtained in the validation study are similar to those in the dataset of 35 948 pregnancies used for development of the algorithm.

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# Multicenter screening for pre-eclampsia by maternal factors and biomarkers at 11–13 weeks' gestation: comparison with NICE guidelines and ACOG recommendations

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**KEYWORDS:** Bayes' theorem; first-trimester screening; mean arterial pressure; placental growth factor; pre-eclampsia; pregnancy-associated plasma protein-A; pyramid of pregnancy care; survival model; uterine artery Doppler

## ABSTRACT

***Objective*** *To compare the performance of screening for pre-eclampsia (PE) based on risk factors from medical history, as recommended by NICE and ACOG, with the method proposed by The Fetal Medicine Foundation (FMF), which uses Bayes' theorem to combine the* a-priori *risk from maternal factors, derived by a multivariable logistic model, with the results of various combinations of biophysical and biochemical measurements.*

***Methods*** *This was a prospective multicenter study of screening for PE in 8775 singleton pregnancies at 11–13 weeks' gestation. A previously published FMF algorithm was used for the calculation of patient-specific risk of PE in each individual. The detection rates (DRs) and false-positive rates (FPRs) for delivery with PE < 32, < 37 and ≥ 37 weeks were estimated and compared with those derived from application of NICE guidelines and ACOG recommendations. According to NICE, all high-risk pregnancies should be offered low-dose aspirin. According to ACOG, use of aspirin should be reserved for women with a history of PE in at least two previous pregnancies or PE requiring delivery < 34 weeks' gestation.*

***Results*** *In the study population, 239 (2.7%) cases developed PE, of which 17 (0.2%), 59 (0.7%) and 180 (2.1%) developed PE < 32, < 37 and ≥ 37 weeks, respectively. Screening with use of the FMF algorithm based on a combination of maternal factors, mean arterial pressure (MAP), uterine artery pulsatility index (UtA-PI) and serum placental growth factor (PlGF) detected 100% (95% CI, 80–100%) of PE < 32 weeks, 75% (95% CI, 62–85%) of PE < 37 weeks and 43% (95% CI, 35–50%) of PE ≥ 37 weeks, at a 10.0% FPR. Screening with use of NICE guidelines detected 41% (95% CI, 18–67%) of PE < 32 weeks, 39% (95% CI, 27–53%) of PE < 37 weeks and 34% (95% CI, 27–41%) of PE ≥ 37 weeks, at 10.2% FPR. Screening with use of ACOG recommendations detected 94% (95% CI, 71–100%) of PE < 32 weeks, 90% (95% CI, 79–96%) of PE < 37 weeks and 89% (95% CI, 84–94%) of PE ≥ 37 weeks, at 64.2% FPR. Screening based on the ACOG recommendations for use of aspirin detected 6% (95% CI, 1–27%) of PE < 32 weeks, 5% (95% CI, 2–14%) of PE < 37 weeks and 2% (95% CI, 0.3–5%) of PE ≥ 37 weeks, at 0.2% FPR.*

***Conclusion*** *Performance of screening for PE at 11–13 weeks' gestation by the FMF algorithm using a combination of maternal factors, MAP, UtA-PI and PlGF, is by far superior to the methods recommended by NICE and ACOG.* Copyright © 2017 ISUOG. Published by John Wiley & Sons Ltd.

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## INTRODUCTION

The traditional approach to screening for pre-eclampsia (PE) is to identify risk factors from maternal demographic characteristics and medical history (maternal factors)[1,2]. In the UK, the National Institute for Health and Care Excellence (NICE) has issued guidelines recommending that women should be considered to be at high risk of developing PE if they have any one high-risk factor or any two moderate-risk factors; high-risk factors are history of hypertensive disease in previous pregnancy, chronic kidney disease, autoimmune disease, diabetes mellitus or chronic hypertension and moderate-risk factors are first pregnancy, age $\geq$ 40 years, interpregnancy interval > 10 years, body mass index (BMI) at first visit of $\geq$ 35 kg/m$^2$ or family history of PE[1]. In the USA, according to the American College of Obstetricians and Gynecologists (ACOG), taking a medical history to evaluate for risk factors is currently the best and only recommended screening approach for PE; risk factors are nulliparity, age > 40 years, BMI $\geq$ 30 kg/m$^2$, conception by *in-vitro* fertilization, history of previous pregnancy with PE, family history of PE, chronic hypertension, chronic renal disease, diabetes mellitus, systemic lupus erythematosus or thrombophilia[2]. Consequently, the approaches recommended by NICE and ACOG essentially treat each risk factor as a separate screening test, with additive detection rate (DR) and screen-positive rate. According to NICE, all high-risk pregnancies should be offered low-dose aspirin. According to ACOG, use of aspirin should be reserved for women with history of PE in two or more previous pregnancies or PE requiring delivery < 34 weeks' gestation[3].

An alternative approach to screening, developed by The Fetal Medicine Foundation (FMF), allows estimation of individual patient-specific risks of PE requiring delivery before a specified gestation, with the use of Bayes' theorem to combine the *a-priori* risk from maternal factors, derived by a multivariable logistic model, with the results of various combinations of biophysical and biochemical measurements[4,5]. In a previous study, we used data from prospective screening in 35 948 singleton pregnancies at 11–13 weeks to develop an algorithm for the calculation of patient-specific risk of PE[5]. Combined screening by maternal factors, mean arterial pressure (MAP), uterine artery pulsatility index (UtA-PI) and serum placental growth factor (PlGF) achieved detection rates (DRs) of delivery with PE < 32, < 37 and $\geq$ 37 weeks of 89%, 75% and 47%, respectively, at a false-positive rate (FPR) of 10%[5]. A limitation of the study is that the performance of screening by a model derived and tested using the same dataset may be overestimated. However, a recent multicenter study in 8775 singleton pregnancies confirmed the validity of the algorithm and reported DRs of 100% (95% CI, 80–100%), 75% (95% CI, 62–85%) and 43% (95% CI, 35–50%) for PE delivering < 32, < 37 and $\geq$ 37 weeks, respectively, at a 10% FPR[6].

The objective of this study was to examine the performance of screening based on risk factors from medical history, as recommended by NICE[1] and ACOG[2,3], using the method proposed by the FMF.

## METHODS

This was a prospective, non-intervention, multicenter study in singleton pregnancies at 11 + 0 to 13 + 6 weeks' gestation in women booking for routine pregnancy care in one of 12 maternity hospitals in five different countries: King's College Hospital, London, UK; Medway Maritime Hospital, Gillingham, UK; Homerton University Hospital, London, UK; North Middlesex University Hospital, London, UK; Southend University Hospital, Essex, UK; Lewisham University Hospital, London, UK; Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain; Hospital Universitario San Cecilio, Granada, Spain; Hospiten Sur, Tenerife, Spain; Centre Hospitalier Universitaire Brugmann, Brussels, Belgium; Attikon University Hospital, Athens, Greece; and Ospedale Maggiore Policlinico, Milan, Italy. The women were screened between February and September 2015 and gave written informed consent to participate in the study, which was approved by the National Health Service Research Ethics Committee in the UK and the Ethics Committee of each participating hospital in other countries. The results from screening were not made available to the patients or their physicians.

Maternal factors were recorded as described previously[4] and MAP and UtA-PI were measured using standardized protocols[7,8]. Serum pregnancy-associated plasma protein-A (PAPP-A) and PlGF concentrations were measured by an automated device (PAPP-A and PlGF 1-2-3$^{TM}$ kits, DELFIA® Xpress random access platform; PerkinElmer Inc. Wallac Oy, Turku, Finland). Measured values of MAP, UtA-PI, PAPP-A and PlGF were expressed as multiples of the median, adjusting for those characteristics found to provide a substantive contribution to the $\log_{10}$-transformed value, including maternal factors, in the prior model[9–12].

The outcome measure was PE, as defined by the International Society for the Study of Hypertension in Pregnancy[13]. Data on pregnancy outcome were collected from the hospital maternity records of the women. The obstetric records of all women with pre-existing or pregnancy-associated hypertension were examined to determine if the condition was PE.

The FMF algorithm was used for the calculation of patient-specific risks of delivery with PE delivering < 32, < 37 and $\geq$ 37 weeks' gestation using maternal factors and various combinations of maternal factors and biomarkers[4,5]. DR, with 95% CI, at a FPR of 10% was estimated. Similarly, the maternal characteristics and medical history of each patient were examined to determine whether they were screen positive or negative according to the NICE[1] and ACOG[2,3] guidelines; the DR, with 95% CI, of delivery with PE delivering < 32, < 37 and $\geq$ 37 weeks' gestation and the FPR were then estimated.

The statistical software package R was used for data analyses[14].

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## RESULTS

In the study population, 239 (2.7%) cases developed PE, of which 17 (0.2%), 59 (0.7%) and 180 (2.1%) developed PE < 32, < 37 and ≥ 37 weeks, respectively, and 8536 cases were without PE. Baseline demographic and clinical characteristics of participants are shown in Table S1.

The performance of screening by the FMF algorithm[4,5] and the methods advocated by NICE[1] and ACOG[2,3] are summarized in Table 1. Combined screening by maternal factors, MAP, UtA-PI and PlGF[4,5] detected 100% (95% CI, 80–100%) of PE < 32 weeks, 75% (95% CI, 62–85%) of PE < 37 weeks and 43% (95% CI, 35–50%) of PE ≥ 37 weeks, at a 10.0% FPR. The receiver–operating characteristics curves are shown in Figure 1.

Screening with use of NICE guidelines[1] detected 41% (95% CI, 18–67%) of PE < 32 weeks, 39% (95% CI, 27–53%) of PE < 37 weeks and 34% (95% CI, 27–41%) of PE ≥ 37 weeks, at a 10.2% FPR. Screening with use of ACOG recommendations[2] detected 94% (95% CI, 71–100%) of PE < 32 weeks, 90% (95% CI, 79–96%) of PE < 37 weeks and 89% (95% CI, 84–94%) of PE ≥ 37 weeks, at a 64.2% FPR. The results of the methods advocated by NICE[1] and ACOG[2,3] are illustrated in Figure 1. Screening based on the ACOG recommendations for use of aspirin[3] detected 6% (95% CI, 1–27%; 1/17) of PE < 32 weeks, 5% (95% CI, 2–14%; 3/59) of PE < 37 weeks and 2% (95% CI, 0.3–5%; 3/180) of PE ≥ 37 weeks, at a 0.2% (19/8536) FPR.

## DISCUSSION

### Main findings

The findings of this prospective multicenter validation study demonstrate that the performance of first-trimester screening for PE by the FMF algorithm, in which the patient-specific risk is derived from a combination of maternal factors, MAP, UtA-PI and PlGF[4,5], is by far superior to the methods advocated by NICE[1] and ACOG[2,3]. In screening by the FMF algorithm, the DRs of delivery with PE < 32, < 37 and ≥ 37 weeks' gestation were 100%, 75% and 43%, respectively, at a FPR of 10.0%. The respective DRs in screening according to NICE guidelines[1] were 41%, 39% and 34%, at a FPR of 10.2%. In the case of ACOG recommendations[2], about two-thirds of the population were classified as screen positive; the DRs of delivery with PE < 32, < 37 and ≥ 37 weeks were 94%, 90% and 89%, respectively, at a FPR of 64.2%. In screening based on the ACOG recommendations for use of aspirin[3], the DRs of delivery with PE < 32, < 37 and ≥ 37 weeks were 6%, 5% and 2%, respectively, at a FPR of 0.2%.

### Study limitations

The main limitation of the study relates to the low incidence of delivery with PE with the inevitable wide confidence intervals obtained for performance of screening. Nevertheless, the values obtained in the validation study are very similar to those in the dataset of 35 948 pregnancies that was used for development of the algorithm[5].

**Table 1** Detection rate of pre-eclampsia (PE) delivering < 32, < 37 or ≥ 37 weeks' gestation in validation dataset using screening algorithm developed by The Fetal Medicine Foundation (FMF)[5] based on maternal factors and combinations of biomarkers, and using recommendations of National Institute of Health and Care Excellence (NICE)[1] and American College of Obstetricians and Gynecologists (ACOG)[2,3]

| | *DR (%) of PE with delivery at:* | | |
|---|---|---|---|
| *Screening method* | *< 32 weeks* | *< 37 weeks* | *≥ 37 weeks* |
| FMF algorithm (FPR = 10.0%) | | | |
| Maternal factors | 53 (28–77) | 41 (28–54) | 37 (30–45) |
| Maternal factors plus: | | | |
| MAP | 71 (44–90) | 47 (34–61) | 37 (30–45) |
| UtA-PI | 82 (57–96) | 61 (47–73) | 39 (32–47) |
| PAPP-A | 59 (33–82) | 47 (34–61) | 37 (30–44) |
| PlGF | 88 (64–99) | 63 (49–75) | 39 (32–46) |
| MAP, UtA-PI | 94 (71–100) | 71 (58–82) | 41 (34–49) |
| MAP, PAPP-A | 76 (50–93) | 49 (36–63) | 40 (33–48) |
| MAP, PlGF | 88 (64–99) | 69 (56–81) | 43 (36–51) |
| UtA-PI, PAPP-A | 82 (57–96) | 66 (53–78) | 40 (33–48) |
| UtA-PI, PlGF | 100 (80–100) | 75 (62–85) | 39 (32–47) |
| PlGF, PAPP-A | 88 (64–99) | 66 (53–78) | 39 (32–47) |
| MAP, UtA-PI, PAPP-A | 94 (71–100) | 69 (56–81) | 42 (35–50) |
| MAP, PAPP-A, PlGF | 88 (64–99) | 69 (56–81) | 43 (36–51) |
| MAP, UtA-PI, PlGF | 100 (80–100) | 75 (62–85) | 43 (35–50) |
| UtA-PI, PAPP-A, PlGF | 100 (80–100) | 75 (62–85) | 38 (31–46) |
| MAP, UtA-PI, PAPP-A, PlGF | 100 (80–100) | 80 (67–89) | 43 (35–50) |
| NICE[1] (FPR = 10.2%) | 41 (18–67) | 39 (27–53) | 34 (27–41) |
| ACOG[2] (FPR = 64.2%) | 94 (71–100) | 90 (79–96) | 89 (84–94) |
| ACOG aspirin[3] (FPR = 0.2%) | 6 (1–27) | 5 (2–14) | 2 (0.3–5) |

Values in parentheses are 95% CI. DR, detection rate; FPR, false-positive rate; MAP, mean arterial pressure; PAPP-A, pregnancy-associated plasma protein-A; PlGF, placental growth factor; UtA-PI, uterine artery pulsatility index.

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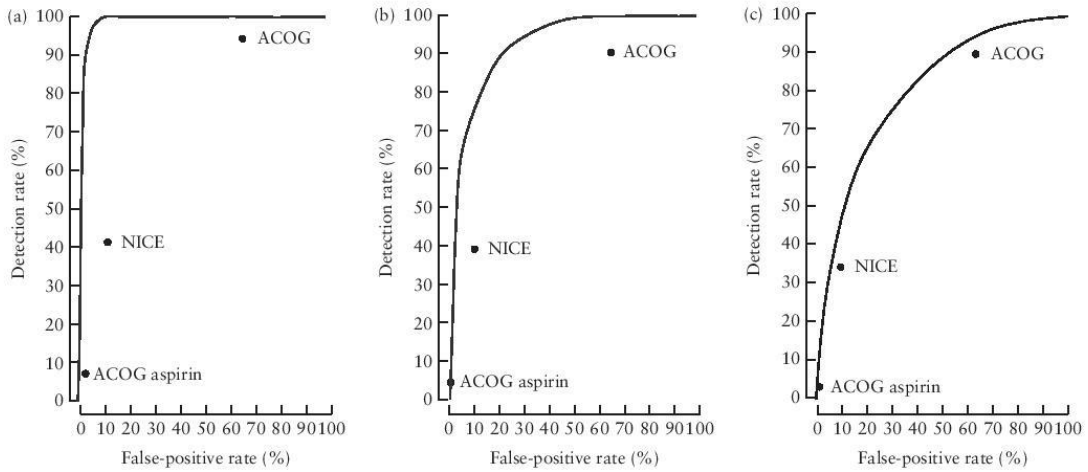


**Figure 1** Receiver–operating characteristics curves for prediction of delivery with pre-eclampsia at < 32 weeks (a), < 37 weeks (b) and ≥ 37 weeks (c) of gestation using The Fetal Medicine Foundation algorithm, combining maternal factors, mean arterial pressure, uterine artery pulsatility index and placental growth factor. Performance of screening using the methods of National Institute for Health and Care Excellence (NICE)[1], American College of Obstetricians and Gynecologists (ACOG)[2] and ACOG for use of aspirin[3] are shown.

### Implications for practice

In a proposed new pyramid of pregnancy care[15], assessment of risk at 11–13 weeks' gestation aims to identify pregnancies at high risk of developing PE and, through pharmacological intervention with such medications as low-dose aspirin, reduce the incidence of these complications[16–18]. Administration of low-dose aspirin from the first-trimester to those at high risk is effective in prevention of preterm, rather than term, PE[18], and the use of the method advocated by the FMF[4,5] is superior to those recommended by NICE[1] and ACOG[2,3] in identifying the group of pregnancies that could benefit from such therapy. According to FMF and NICE, about 10% of the pregnant population would receive low-dose aspirin and this population would contain 75% of those that will develop preterm PE if selection of the high-risk group was based on the FMF algorithm and only 39% if selection was based on the NICE guidelines. In the case of the ACOG recommendations, 0.2% of the population would receive aspirin and only 5% of cases of preterm PE that would potentially benefit from such therapy would be targeted.

The methods of NICE[1] and ACOG[2] treat each maternal factor as a separate screening test with additive DR and FPR. In the FMF method, use of a multivariable logistic model to define the prior risk attributes the appropriate relative importance to each maternal factor and allows estimation of the patient-specific risk of PE requiring delivery before a specified gestation[4]. The prior risk can then be adjusted according to the results of biophysical and biochemical testing[5]. The software for such estimation of prior and adjusted risk is freely available (www.fetalmedicine.com). Recording of maternal history and measurement of blood pressure are universally carried out as part of routine pregnancy care; measurement of MAP requires adherence to a protocol[7] but can be undertaken by healthcare assistants after minimal training, using inexpensive equipment and taking a few minutes to perform. Measurement of UtA-PI requires specific training by sonographers and quality assurance of their results[8]; nevertheless, this test can be undertaken within a few minutes by the same sonographer and machine as part of the routine first-trimester scan. Measurement of serum PlGF can be undertaken on the same machine as free β-human chorionic gonadotropin and PAPP-A, which are widely used in screening for Down syndrome.

## ACKNOWLEDGMENT

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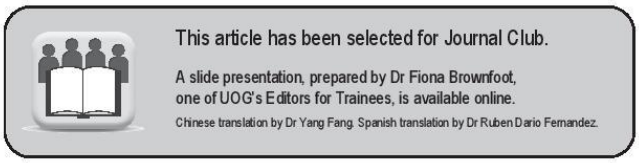
## SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

**Table S1** Characteristics of women with normal singleton pregnancy and of those who developed pre-eclampsia (PE) with delivery < 32 weeks, < 37 weeks or ≥ 37 weeks

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# CHAPTER 4 DISCUSSION & CONCLUSION

## 4.1 Development of the competing risks model

The competing risks model is a new approach for early screening for PE achieved by combining the prior risk, derived from the maternal demographics and medical history, with the biophysical markers uterine artery PI and MAP and the biochemical markers PAPP-A and PlGF. In this survival time model, the gestation at the time of delivery for PE is treated as a continuous rather than a categorical variable. This approach has the advantage of facilitating the creation a more efficient screening model, as opposed to using separate models for early and/or late PE. To further strengthen this argument, our data support this hypothesis and model as the distribution of the MoMs of the above markers is linear with gestational age in pregnancies with PE. One such theory could be that PE is a single pathophysiological disorder with a wide spectrum of severity manifested in gestational age at which delivery becomes necessary for maternal and or fetal indications. In this way, the more severe the disease, *i.e.* the earlier delivery is required, the worse the MoMs are at 11-13 weeks' gestation.

In this study, there were 1,058 cases of PE with an overall incidence of 2.9%, which is consistent with the incidence quoted in the literature (WHO, 2005; Knight *et al*., 2014). 27.6% of these cases required delivery before 37 weeks' gestation resulting in an incidence of preterm PE of 0.8%. All of the biomarkers showed a larger separation from the norm in cases of early PE than in those that occurred at later gestations. This observation is reflected in their detection of early rather than in late disease. This is in keeping with the 'broken stick' statistical model. The regression slopes of the biomarkers tend toward a normal MoM with time (advancing gestational age) but for most cases in reality, this is not observed as the woman will have delivered before this occurs. However, the regression lines for the $\log_{10}$ MoMs values of the uterine artery PI and PAPP-A intersect 1 when delivery occurs close to term and consequently show little or no discriminatory power between affected and unaffected cases beyond 40 weeks' gestation.

The performance of each biomarker in combination with maternal factors was superior to that of screening by maternal factors alone (See chapter 3, page 78, table 4). PlGF combined with maternal characteristics gave the best DR of all the biomarkers and PAPP-A made the least contribution. However, by combining 2 or more biomarkers, better detection rates were achieved than by using individual biomarkers, with the exception of PAPP-A. This biomarker did not provide any significant improvement of detection rates (DR) when combined with PlGF, presumably due to the fact that they are both markers of placental inefficiency so there is a

considerable overlap in their ability to detect PE. In screening for PE requiring delivery before 37 weeks' gestation the detection rate, at a 10% FPR, was 49% by maternal characteristics and with the addition of the biomarkers, this increased to 75%. The detection rate for PE requiring delivery after 37 weeks' gestation was 47% with a FPR of 10%.

Similar to screening for Down's syndrome, the FPR and DR of PE are influenced by the characteristics of the study population and for a given risk cut-off, they are both higher in nulliparous than in parous women and in those of Afro-Caribbean compared with Caucasian racial origin. Consequently, comparison of the performance of screening using these algorithms between studies requires the appropriate adjustments for the characteristics of the population under investigation.

### 4.2 The validation study

Having established a working model, the subsequent study validated this model in 12 different hospitals across Europe. This prospective multicentre validation study demonstrated not only, that implementing an international first trimester screening programme was feasible, but also showed that the performance of such a programme was comparable to that of our original model. At a FPR of 10%, the prospective DRs of screening for PE using maternal factors, uterine artery PI, MAP, and PlGF were 100%, 75%, and 47% for PE requiring delivery before 32, 37, and after 37 weeks' gestation respectively. These findings confirm that first trimester screening for PE identifies a high proportion of cases that will develop early onset disease but the detection of term PE still remains a challenge.

### 4.3 The comparison study

The current recommended approach to screening for PE is based on using maternal demographics and medical history to identify specific risk factors. In the UK, NICE advocate that women should be started on 75mg of aspirin daily until the birth of the baby if they have one high risk factor or two moderate risk factors (See table 1.5). In the United States, ACOG recommend taking a medical history to evaluate the woman's risk of PE. They suggest only prescribing aspirin (80mg) to women with a history of PE in two or more previous pregnancies or to those who required delivery in a prior pregnancy before 34 weeks' gestation as a result of PE. The approach suggested by these two bodies, effectively treats each risk factor as a separate screening test.

This study examined the performance of screening based on risk factors from the medical history as recommended by NICE and ACOG against our competing risks model. In comparison to our model, the recommended approach by NICE performed significantly lower

with a DR of 41% and 39% for PE requiring delivery before 32 and 37 weeks' gestation, respectively, at a FPR of 10.2%. The ACOG approach had high DRs of 94% and 90% for PE <32 and <37 weeks' gestation but with a FPR of 64.2% and based on their recommendation for prescribing low dose aspirin, we found their DRs would be 6%, 5%, and 2% for PE requiring delivery before 32 and 37 weeks' gestation and after 37 weeks respectively.

### 4.4 Implications for clinical practice

It was first realised that aspirin was associated with a lower risk of PE in high-risk nulliparous women in 1979 (Crandon and Isherwood). Another study randomised high risk women to either 150mg of aspirin and 300mg of dipyridamole or no treatment (Beaufils *et al.,* 1985) and found the incidence of PE in the treatment group significantly less compared to the control group. Since then, there have been many studies investigating this subject with no definitive conclusion. The controversy is a result of the very heterogeneous nature in the methodology used in these studies with respect to population, screening method, dose and time of initiation of aspirin and definitions of PE (Duley *et al*., 2006.) Recent meta-analyses have suggested that the effect of aspirin in the prevention of PE is gestation-dependent (Bujold *et al.,* 2010) and that a 50% reduction in the incidence of PE is achievable if aspirin is commenced before 16 weeks' gestation, and particularly the incidence of severe and preterm forms of the disease (Roberge *et al.,* 2012a and 2012b). These observations were, however, questioned as in these meta-analyses, the number of women given aspirin before 16 weeks' gestation was small in comparison to those whom commenced aspirin after 16 weeks' gestation and there were no major randomised controlled trials confirming such findings. These criticisms were answered by two major studies. Park *et al.* (2015) performed a retrospective cohort analysis of 7,783 patients showing that a combination of first-trimester PE screening combined with 150 mg of aspirin daily in high-risk women reduced the risk of preterm PE by 55% in their population (Park *et al*., 2015). More recently, Rolnik *et al.* published the results of the ASPRE trial that screened 26,941 patients for PE at 11 to 13 weeks' gestation using the competing risks model described in chapter 3 and randomised 1,776 women at high risk of PE. They found a significant 62% reduction on preterm PE when aspirin was given at a 150mg dose and initiated before 16 weeks' gestation. Whilst the effect on term PE was minimal, there was an overall 30% reduction in all forms of disease (Rolnik *et al.,* 2017).

Historically, the current approach to prenatal care is based on a pyramid of care that involved a first visit at 12 weeks' gestation and a high concentration of visits in the second and, mainly, in the third trimester of pregnancy. Since the early 1990's, however, there has been a shift towards attempting to predict pregnancy complications in the first trimester (Nicolaides, 2011a). Early identification of high-risk women allows appropriate counselling regarding

changes in lifestyle (such as healthy diet, exercising, weight gain recommendations) and early initiation of other preventive measures (Nicolaides, 2011a).

In a proposed new pyramid of pregnancy care (Nicolaides, 2011b) an integrated antenatal visit at $11^{+0}$ to $13^{+6}$ weeks' gestation at the same time as the well-established process of first trimester screening for chromosomal abnormalities, can provide a framework for population-based screening and preventive interventions. This study provides evidence that screening for PE using biochemical and biophysical markers combined with maternal factors, can be added to this first trimester routine visit. For units wishing to implement this algorithm, the choice of which combination of markers used, will depend on the feasibility of its introduction, as well as, the healthcare costs. The measurement of the MAP can be easily performed by any member of the healthcare team using automated, pregnancy validated, blood pressure machines. A strict protocol is required to be followed and a mechanism for careful surveillance of the quality of the measurements should be put in place. The uterine artery PI can be performed by the same sonographer who carries out the first trimester scan following specific training to ensure correct measurements. In many countries, the infrastructure is already in place for the measurement of PAPP-A in centres that provide routine first trimester screening for aneuploidies. The PlGF measurement can be performed on the same blood sample by the same machine as PAPP-A, *albeit* at an extra cost.

The findings of the above studies demonstrate that in first-trimester screening for PE the performance of the test is better for early rather than late disease. This is particularly relevant as the objective of early screening is to identify the high-risk group that may benefit from, the now, proven therapeutic interventions, aspirin 150mg initiated before 16 weeks' gestation, that reduce the prevalence of preterm PE.

### 4.5 Strengths and Limitations

These studies included a large population of pregnant women who attended for routine care in a gestational age range widely used for the assessment of risk of chromosomal abnormalities. The adherence to the strict protocols and appropriately trained personnel was a strength of these studies, which contributed to the high quality of data obtained. Another strength was the use of automated machines to provide accurate measurements of markers within 40 minutes of sampling. Importantly, the biomarkers were expressed in MoMs after adjustment for the factors that affect their measurement. This approach adjusted for additional factors that influence the biomarkers in the algorithm and consequently the risk calculation. The use of Bayes' theorem to combine the prior risk with biomarkers to estimate patient specific risks contributed significantly to the performance of the screening. All patient medical records,

with a suspected diagnosis of hypertensive disease in pregnancy and IUGR, where thoroughly reviewed to ensure an accurate diagnosis.

A limitation of the competing risks model study is that it was derived and tested with the use of the same dataset. One criticism of this approach is that it can lead to an overestimation and overfitting. Overfitting occurs when a model corresponds very closely or exactly to a particular set of data. It may therefore fail to accommodate at fit additional data or even predict future observations reliably. To limit this potential bias, a five-fold cross validation was used. A major strength in this study is the prospective validation of this model using a new data set.

The main limitation of the validation and comparison study relates to the low incidence of delivery with PE with the inevitable wide confidence intervals obtained for performance of screening. However, the values and detection rates obtained from the validation study are very similar to those in the dataset of the competing risks model that was used for the development of the model.

## 4.6 Future studies

First trimester screening for preterm PE performs well and can successfully identify a significant proportion of women that will develop this disorder. However, owing to the complex nature of this disease, no single predictive marker exists. Identifying potentially superior predictive markers is the focus of ongoing research. Additionally, the first trimester prediction of term PE is still poor, raising the hypothesis that the mechanism of disease is different in these cases (Melchiorre *et al*. 2011). Early PE is associated with a very high-risk of maternal and perinatal morbidity and mortality, and late PE and its consequences have a significant impact on public health since this condition is more common. Aspirin significantly reduces the incidence of preterm PE but has little or no effect on term disease. This may be due to the poor performance of actually detecting term disease or aspirin is converting what would have been early PE to late PE. Better methods of prediction and prevention of late PE are needed.

### 4.6.1 Proteomics

Mass spectrometry-based proteomics has been an exciting breakthrough and in theory could provide vital information necessary for understanding the mechanisms of complex human diseases, such as PE, through the discovery of novel biomarkers. It is an automated technique that can detect large numbers of peptides from biological fluids, thus yielding a proteomic signature that can be distinguished from matched control cases.

A number of previously published studies have verified that proteomics can identify peptides specific to preeclampsia (Pecks *et al.,* 2010*,* Blankly *et al.,* 2013). Myers *et al.* (2013) externally validated their proteomic signature with plasma samples taken at 22 and 26 weeks of gestation. They described a prediction model with a DR of 54% and 80% with a 20% positive predictive value (PPV) for PE and preterm PE respectively. However, there are very limited data available on first trimester screening for PE using proteomics. One study used quantitative proteomic (iTRAQ) analysis of maternal plasma samples taken at 12 weeks' gestation and found that there were 31 proteins upregulated and 20 proteins downregulated in patients that subsequently developed PE, demonstrating the potential for the development of a first trimester screening model for PE using proteomics (Liu *et al.,* 2011). This could provide an interesting avenue of exploration that yields biomarkers that could be used alone or combined with existing screening risk models.

### 4.6.2 Metabolomics

Metabolites are the intracellular molecules that undergo transformation when metabolised and in effect, provide a blueprint log of its cellular biochemistry. Metabolomics is the detection and semi-quantitation of low molecular weight metabolites present in cells, tissues or body fluids, using proton nuclear magnetic resonance ($^1$H NMR) spectroscopy or mass spectrometry. Recently, there has been emerging interest in this approach to predict PE. First trimester prediction of PE using metabolomic markers have been published (Bahado-Singh *et al.,* 2012 and 2013). They reported such an approach appeared highly predictive of early PE with an estimated detection rate of 75.9%, at a false-positive rate (FPR) of 4.9%. This was further improved by the addition of uterine artery Doppler PI and fetal crown-rump length (CRL) and with an estimated detection rate of 82.6%, at a FPR of 1.6%. Metabolomic prediction for late PE did not perform as well. However, a complex mix of potential pathways for disease development was identified, which could lead to exciting new therapeutic options in the future. This approach affords more than just mere biomarker development. It may also generate significant insights into the pathophysiology of this disorder.

### 4.6.3 Fetal haemoglobin

Extracellular fetal haemoglobin (HbF) was first suggested to be implicated in the pathogenesis of PE in 2008 by Centlow *et* al. They found an upregulation of HbF genes and accumulation of extracellular HbF in the vascular lumen in PE placentas. HbF has been suggested to be a causative factor of PE (Hansson *et al*., 2013). More recently, HbF and the heme-scavenger a1- microglobulin (A1M) were found to be significantly raised in patients with a diagnosis of PE (Anderson *et al*., 2015) raising the possibility of potentially two new biomarkers. Further larger studies are awaited to see whether they can be potential predictive markers and in what capacity.

## 4.7 Conclusions

Using a competing risks model, an algorithm that incorporated maternal characteristics with biophysical and biochemical markers, was developed to screen pregnant women from 11 to 13 weeks' gestation for PE. This study, based on 35,948 singleton pregnancies, had a DR of 75% and 47% for PE requiring delivery before and after 37 weeks' gestation respectively, at a FPR of 10% (O'Gorman *et al.,* 2016).

In our prospective European multicentre validation study, we screened 9,041 women with singleton pregnancies. The same protocols were employed at all centres ensuring homogeneity of the methodology and quality of the results. This study found similar DR to those predicted from the model (DR of PE before and after 37 weeks' gestation 75% and 43% respectively at a FPR of 10%), thus validating the prediction algorithm and illustrating its reproducibility at external centres (O'Gorman *et al.,* 2017a).

The same data from the validation study was used to examine the performance of screening based on risk factors from the patient's medical history as recommended by NICE and ACOG. In comparison to the DRs from our competing risks model, the screening approach endorsed by NICE performed significantly lower for the detection of preterm PE with DRs of 39% and 34% of PE before and after 37 weeks' gestation respectively at a FPR of 10%. While our model detected 100% of PE requiring delivery before 32 weeks' gestation, the recommended approach by NICE detected just 41% at the same FPR (O'Gorman *et al.,* 2017b).

The DR were higher in the screening approach suggested by ACOG 94%, 90% and 89% for PE <32, <37 and $\geq$37 weeks' gestation respectively but had a higher FPR of 64.2%. Interestingly, based on their recommendations as to whom aspirin should be given to, ACOG would only detect 6%, 5%, and 2% of women developing PE requiring delivery before 32 and 37 weeks' gestation and after 37 weeks' gestation respectively (O'Gorman *et al.,* 2017b).

In conclusion, the studies included in the papers of Chapter 3 demonstrate that effective screening for PE at 11 to 13 weeks' gestation is feasible internationally. We developed a model that has a high DR for PE requiring delivery before 37 weeks' gestation. The algorithm was prospectively validated and subsequently proven to perform better than, the screening models endorsed by the NICE and the ACOG. This effective screening model is perfectly timed to facilitate the initiation of aspirin, which has been proven to significantly decrease the incidence of preterm PE.

## RESEARCH STUDY
## EARLY PREDICTION OF PREGNANCY COMPLICATIONS

We are looking for new ways through scientific research to improve the care of pregnant women and their unborn babies. As part of this work, we are inviting all women that attend our unit for their routine scans at 11-13, 20-23, and 32-36 weeks' to participate in a large study on detection and prediction of pregnancy complications such as preeclampsia (high blood pressure of pregnancy) and premature birth.

Pre-eclampsia and premature birth are two important complications of pregnancy which can have serious implications for mother and baby. These problems can affect any pregnant woman, irrespective of previous healthy pregnancies and irrespective of how healthy the mother is.

Our aim is to try and identify the women who are at high risk of developing such pregnancy complications and to do so as early in pregnancy as possible.

**Routine care**

Each visit at 11-13, 20-23 and 32-36 weeks will include:

* Ultrasound examination of the fetus to diagnose major abnormalities and to assess blood flow to the uterus and placenta.

* Measurement of your weight, height and blood pressure.

The visit at 11-13 weeks will also include assessment of your risk for Down's syndrome and other chromosomal defects from ultrasound markers and a blood test. The visit at 20-23 weeks will also include measurement of the length of the cervix (neck of the womb). The visit at 32-36 weeks' will also include ultrasound examination of blood flow in the cord and vessels of the baby to assess fetal well-being.

**What will happen to me if I take part in the research study?**

This will involve the following:

* Assess blood flow changes in you uterus by ultrasound and your heart in a manner similar to measurement of a heart trace (an ECG with 4 stickers).

* Saving some of the blood that we take routinely from you to determine the risk for Down's syndrome at 11-13 weeks. We will also collect a blood sample at 20-23, and 32-36 weeks. These samples will be stored for analysis in the future, after the end of your pregnancy. The results will not affect the management of your pregnancy.

* We will collect a urine sample at each visit. These samples will be stored for analysis in the future, after the end of your pregnancy. The results will not affect the management of your pregnancy.

**Why have I been chosen?**

All pregnant women attending our unit for their scans are welcome to take part in this study.

**Do I have to take part?**

It is up to you to decide whether you would like to take part. If you decide to take part you will be given this information sheet to keep and will be asked to sign a consent form. Once you have decided to take part you are still free to withdraw at any time without giving any reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

**What are the possible benefits of taking part?**

The information from analysis of the samples will have no direct benefit to your current pregnancy. The information we get from the study may help us to help you and/or other women in the future.

**What are the possible disadvantages and risks of taking part?**

There are no disadvantages or risks to you or your baby from allowing us to store some of the blood sample we collect.

**Will my taking part in this study be kept confidential?**

Yes. All the information about your participation in this study will be kept confidential.

**What if I want to complain?**

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions [see below]. If you remain unhappy and wish to complain formally, you can contact the Patient Advice and Liaison Service (PALS) at 02032993601 or email kch-tr.PALS@nhs.net.

**What will happen to the results of the research study?**

The results will be published in medical journals and perhaps also in the press. You may request a copy of any published documents in relation to the study. You will not be identified in any of these reports.

**Who is organising and funding the research?**

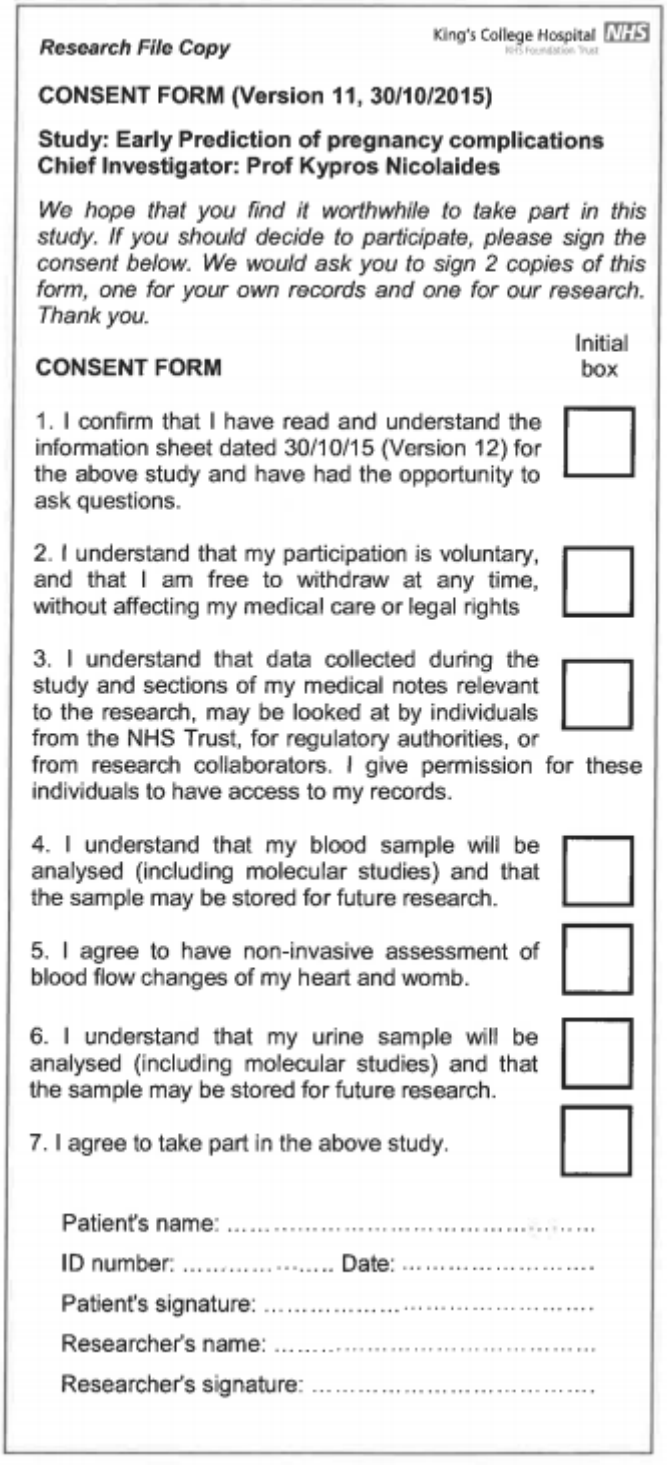
This research is carried out by the team of Professor Kypros Nicolaides and it is funded by the Fetal Medicine Foundation (UK registered charity).

**Contact for further information**

Dr Liona Poon 02032999000 or 02032993246 (option 3)

**Version 12, 30/10/2015**

**Appendix 2** Patient consent form – The competing risks model



*Research File Copy*

King's College Hospital NHS

**CONSENT FORM (Version 11, 30/10/2015)**

**Study: Early Prediction of pregnancy complications**
**Chief Investigator: Prof Kypros Nicolaides**

*We hope that you find it worthwhile to take part in this study. If you should decide to participate, please sign the consent below. We would ask you to sign 2 copies of this form, one for your own records and one for our research. Thank you.*

**CONSENT FORM** — Initial box

1. I confirm that I have read and understand the information sheet dated 30/10/15 (Version 12) for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary, and that I am free to withdraw at any time, without affecting my medical care or legal rights ☐

3. I understand that data collected during the study and sections of my medical notes relevant to the research, may be looked at by individuals from the NHS Trust, for regulatory authorities, or from research collaborators. I give permission for these individuals to have access to my records. ☐

4. I understand that my blood sample will be analysed (including molecular studies) and that the sample may be stored for future research. ☐

5. I agree to have non-invasive assessment of blood flow changes of my heart and womb. ☐

6. I understand that my urine sample will be analysed (including molecular studies) and that the sample may be stored for future research. ☐

7. I agree to take part in the above study. ☐

Patient's name: .....

ID number: ..... Date: .....

Patient's signature: .....

Researcher's name: .....

Researcher's signature: .....

# **Appendix 3** Patient information leaflet – The validation study

King's College Hospital NHS
NHS Foundation Trust

UCL Clinical Trials Unit

**Patient information leaflet**

***ASPRE Screening Quality Study Version 1.0 16.10.2014***

**Screening for pre-eclampsia (high blood pressure)**
**Screening Quality Study**

You are being invited to take part in a research study. Before you decide whether to take part it is important for you to understand why the research is being done and what it will involve. Please take your time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. If you have any questions or require any further information please contact the trial co-ordinator at King's College Hospital, Dr Leona Poon, Dr Daniel Rolnik, or Dr Neil O'Gorman.

**What is the purpose of the study?**

The placenta (afterbirth) is responsible for providing food and oxygen to the fetus. When there is a problem with the function of the placenta the fetus may not grow well and the mother can develop high blood pressure (pre-eclampsia). Placental problems affect about 10% of pregnancies. The consequences are usually minor but occasionally they can be serious both for the mother and the baby.

The flow of blood through the uterine arteries (blood vessels which supply blood to the placenta), your blood pressure and the measurement in your blood of proteins produced by the placenta are important in determining whether you are at increased risk of developing pre-eclampsia or problems with the growth of the baby.

Our aim is to establish standards for screening for pre-eclampsia in early pregnancy.

You can choose not to have the Down's syndrome test even if you are coming for the screening but you might want to take part in the screening quality study for pre-eclampsia.

**Why have I been chosen?**

All women with a singleton pregnancy attending for their routine screening for Down's syndrome are invited to take part in the study.

**Do I have to take part?**

It is up to you whether or not to take part in the study. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the care you receive.

**What will happen to me if I take part?**

During your visit at 11-13 weeks, we will measure your weight, height and blood pressure, and assess blood flow to the uterus and placenta during your routine ultrasound scan. We will record these results and the outcome of your pregnancy but your pregnancy care will not change from the existing standard of care in place at your hospital.

We will ask for your permission to save some of your blood sample which will be stored for further research such as measuring factors that may be implicated in pregnancy complications in the future.

**What do I have to do?**

There are no other restrictions as to what you can or cannot do.

**What if there is a problem?**

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions [Dr Neil O'Gorman or Dr Daniel Rolnik via switchboard 020 3299 9000]. If you remain unhappy and wish to complain formally, you can contact the local Patient Advice and Liaison Service (PALS) at 020 3299 3601 or email kch-tr.PALS@nhs.net.

University College London (the trial Sponsor) has insurance arrangements for this study. In the unlikely event that you are injured by taking part in this trial, compensation may be available. In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against University College London Hospitals but you may have to pay your legal costs.

**Will my records be kept confidential?**

All information collected about you during this study will be kept strictly confidential. Any information that leaves the hospital will have your name and address removed so that you cannot be recognised from it.

**What will happen to the results of the study?**

Once the study is complete, the results will be reviewed and used to inform a full-scale trial combining a screening study and randomised controlled trial. You will not be identified in any reports.

**Who is organising and funding the research?**

Funding to conduct the trial is provided by The European Commission Seventh Framework Programme FP7 (#601852).

**Who has reviewed the study?**

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by NRES Committee London – Fulham Research Ethics Committee.

The Sponsor for the study is University College London.

## **Appendix 4** Patient consent form – The validation study

King's College Hospital NHS
NHS Foundation Trust

UCL Clinical Trials Unit

**Patient consent form**
***ASPRE Screening Quality Study Version 1.0 16.10.14***

**Screening for pre-eclampsia (high blood pressure)**
**Screening Quality Study**

**Consent form**

**Please initial box**

I confirm that I have read and understand the patient information sheet (Version _____ Dated _____) for the above study and have had the opportunity to ask questions and have had these answered satisfactorily. ☐

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

I agree to participate in the screening study for pre-eclampsia. ☐

I agree to have my blood sample stored for an indefinite period for future research on pregnancy complications. ☐

I understand that relevant sections to my medical notes and data collected during the study may be looked at by authorised individuals, from regulatory authorities or from Sponsor or NHS trust where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

I agree to have my anonymised data stored for more than 10 years. ☐

**Consent for screening quality study:**

Name of patient..... Signature of patient..... Date.....

Researcher..... Signature..... Date.....

Witness..... Signature..... Date.....

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